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(54) Title: HUMAN DNA MISMATCH REPAIR PROTEINS

(57) Abstract

The present invention discloses three human DNA repair proteins and DNA (RNA) encoding such proteins and a procedure for producing such proteins by recombinant techniques. One of the human DNA repair proteins, hMLH1, has been mapped to chromosome 3 while hMLH2 has been mapped to chromosome 2 and hMLH3 has been mapped to chromosome 7. The invention provides methods to diagnose alterations in the hMLH1, hMLH2 and hMLH3 genes.

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HUMAN DNA MISMATCH REPAIR PROTEINS

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptides of the present invention are human homologs of the prokaryotic *mutL4* gene and are hereinafter referred to as hMLH1, hMLH2 and hMLH3.

In both prolaryotes and eukaryotes, the DNA mismatch repair gene plays a prominent role in the correction of errors made during DNA replication and genetic recombination. The *E.coli* methyl-directed DNA mismatch repair system is the best understood DNA mismatch repair system to date. In *E.coli*, this repair pathway involves the products of the mutator genes *mutS*, *mutL*, *mutH*, and *uvrD*. Mutants of any one of these genes will reveal a mutator phenotype. *MutS* is a DNA mismatch-binding protein which initiates this repair process, *uvrD* is a DNA helicase and *MutH* is a latent

endonuclease that incises at the unmethylated strands of a hemi-methylated GATC sequence. *MutL* protein is believed to recognize and bind to the mismatch-DNA-MutS-MutH complex to enhance the endonuclease activity of *MutH* protein. After the unmethylated DNA strand is cut by the *MutH*, single-stranded DNA-binding protein, DNA polymerase III, exonuclease I and DNA ligase are required to complete this repair process (Modrich P., Annu. Rev. Genetics, 25:229-53 (1991)).

Elements of the *E.coli MutLHS* system appears to be conserved during evolution in prokaryotes and eukaryotes. Genetic study analysis suggests that *Saccharomyces cerevisiae* has a mismatch repair system similar to the bacterial *MutLHS* system. In *S. cerevisiae*, at least two *MutL* homologs, *PMS1* and *MLH1*, have been reported. Mutation of either one of them leads to a mitotic mutator phenotype (Prolla et al, Mol. Cell. Biol. 14:407-415 (1994)). At least three *MutS* homologs have been found in *S.cerevisiae*, namely *MSH1*, *MSH2*, and *MSH3*. Disruption of the *MSH2* gene affects nuclear mutation rates. Mutants in *S. cerevisae*, *MSH2*, *PMS1*, and *MLH1* have been found to exhibit increased rates of expansion and contraction of dinucleotide repeat sequences (Strand et al., Nature, 365:274-276 (1993)).

It has been reported that a number of human tumors such as lung cancer, prostate cancer, ovarian cancer, breast cancer, colon cancer and stomach cancer show instability of repeated DNA sequences (Han et al., Cancer, 53:5087-5089 (1993); Thibodeau et al., *Science* 260:816-819 (1993); Risinger et al., Cancer 53:5100-5103 (1993)). This phenomenon suggests that lack of the DNA mismatch repair is probably the cause of these tumors.

Little was known about the DNA mismatch repair system in humans until recently, the human homolog of the *MutS* gene was cloned and found to be responsible for hereditary nonpolyposis colon cancer (HNPCC), (Fishel et al., Cell, 75:1027-1038 (1993) and Leach et al., Cell, 75:1215-1225

(1993)). HNPCC was first linked to a locus at chromosome 2p16 which causes dinucleotide instability. It was then demonstrated that a DNA mismatch repair protein (*MutS*) homolog was located at this locus, and that C-->T transitional mutations at several conserved regions were specifically observed in HNPCC patients. Hereditary nonpolyposis colorectal cancer is one of the most common hereditable diseases of man, affecting as many as one in two hundred individuals in the western world.

It has been demonstrated that hereditary colon cancer can result from mutations in several loci. Familial adenomatosis polyposis coli (APC), linked to a gene on chromosome 5, is responsible for a small minority of hereditary colon cancer. Hereditary colon cancer is also associated with Gardner's syndrome, Turcot's syndrome, Peutz-Jaeghers syndrome and juvenile polyposis coli. In addition, hereditary nonpolyposis colon cancer may be involved in 5% of all human colon cancer. All of the different types of familial colon cancer have been shown to be transmitted by a dominant autosomal mode of inheritance.

In addition to localization of HNPCC, to the short arm of chromosome 2, a second locus has been linked to a predisposition to HNPCC (Lindholm, et al., Nature Genetics, 5:279-282 (1993)). A strong linkage was demonstrated between a polymorphic marker on the short arm of chromosome 3 and the disease locus.

This finding suggests that mutations on various DNA mismatch repair proteins probably play crucial roles in the development of human hereditary diseases and cancers.

HNPCC is characterized clinically by an apparent autosomal dominantly inherited predisposition to cancer of the colon, endometrium and other organs. (Lynch, H.T. et al., Gastroenterology, 104:1535-1549 (1993)). The identification of markers at 2p16 and 3p21-22 which were linked to disease in selected HNPCC kindred unequivocally

established its mendelian nature (Peltomaki, P. et al., Science, 260:810-812 (1993)). Tumors from HNPCC patients are characterized by widespread alterations of simple repeated sequences (microsatellites) (Aaltonen, L.A., et al., Science, 260:812-816 (1993)). This type of genetic instability was originally observed in a subset (12 to 18% of sporadic colorectal cancers (Id.). Studies in bacteria and yeast indicated that a defect in DNA mismatch repair genes can result in a similar instability of microsatellites (Levinson, G. and Gutman, G.A., Nuc. Acids Res., 15:5325-5338 (1987)), and it was hypothesized that deficiency in mismatched repair was responsible for HNPCC (Strand, M. et al., Nature, 365:274-276 (1993)). Analysis of extracts from HNPCC tumor cell lines showed mismatch repair was indeed deficient, adding definitive support to this conjecture (Parsons, R.P., et al., Cell, 75:1227-1236 (1993)). As not all HNPCC kindred can be linked to the same loci, and as at least three genes can produce a similar phenotype in yeast, it seems likely that other mismatch repair genes could play a role in some cases of HNPCC.

hMLH1 is most homologous to the yeast mutL-homolog yMLH1 while hMLH2 and hMLH3 have greater homology to the yeast mutL-homolog yPMS1 (hMLH2 and hMLH3 due to their homology to yeast PMS1 gene are sometimes referred to in the literature as hPMS1 and hPMS2). In addition to hMLH1, both the hMLH2 gene on chromosome 2q32 and the hMLH3 gene, on chromosome 7p22, were found to be mutated in the germ line of HNPCC patients. This doubles the number of genes implicated in HNPCC and may help explain the relatively high incidence of this disease.

In accordance with one aspect of the present invention, there are provided novel putative mature polypeptides which are hMLH1, hMLH2 and hMLH3, as well as biologically active and diagnostically or therapeutically useful fragments,

analogs and derivatives thereof. The polypeptides of the present invention are of human origin.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules encoding such polypeptides, including mRNAs, DNAs, cDNAs, genomic DNA as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof.

In accordance with still another aspect of the present invention there are provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to hMLH1, hMLH2 and hMLH3 sequences.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques which comprises culturing recombinant prokaryotic and/or eukaryotic host cells, containing an hMLH1, hMLH2 or hMLH3 nucleic acid sequence, under conditions promoting expression of said protein and subsequent recovery of said proteins.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptide, or polynucleotide encoding such polypeptide, for therapeutic purposes, for example, for the treatment of cancers.

In accordance with another aspect of the present invention there is provided a method of diagnosing a disease or a susceptibility to a disease related to a mutation in the hMLH1, hMLH2 or hMLH3 nucleic acid sequences and the proteins encoded by such nucleic acid sequences.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotides encoding such polypeptides, for *in vitro* purposes related to scientific research, synthesis of DNA and manufacture of DNA vectors.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figure 1 illustrates the cDNA sequence and corresponding deduced amino acid sequence for the human DNA repair protein hMLH1. The amino acids are represented by their standard one-letter abbreviations. Sequencing was performed using a 373 Automated DNA sequencer (Applied Biosystems, Inc.). Sequencing accuracy is predicted to be greater than 97% accurate.

Figure 2 illustrates the cDNA sequence and corresponding deduced amino acid sequence of hMLH2. The amino acids are represented by their standard one-letter abbreviations.

Figure 3 illustrates the cDNA sequence and corresponding deduced amino acid sequence of hMLH3. The amino acids are represented by their standard one-letter abbreviations.

Figure 4. Alignment of the predicted amino acid sequences of S. cerevisiae PMS1 (yPMS1), with the hMLH2 and hMLH3 amino acid sequences using MACAW (version 1.0) program. Amino acid in conserved blocks are capitalized and shaded on the mean of their pair-wise scores.

Figure 5. Mutational analysis of hMLH2. (A) IVSP analysis and mapping of the transcriptional stop mutation in HNPCC patient CW. Translation of codons 1 to 369 (lane 1), codons 1 to 290 (lane 2), and codons 1 to 214 (lane 3). CW is translated from the cDNA of patient CW, while NOR was translated from the cDNA of a normal individual. The arrowheads indicate the truncated polypeptide due to the potential stop mutation. The arrows indicate molecular weight markers in kilodaltons. (B) Sequence analysis of CW indicates a C to T transition at codon 233 (indicated by the arrow). Lanes 1 and 3 are sequence derived from control

patients; lane 2 is sequence derived from genomic DNA of CW. The ddA mixes from each sequencing mix were loaded in adjacent lanes to facilitate comparison as were those for ddC, ddD, and ddT mixes.

Figure 6. Mutational analysis of hMLH3. (A) IVSP analysis of hMLH3 from patient GC. Lane GC is from fibroblasts of individual GC; lane GCx is from the tumor of patient GC; lanes NOR1 and 2 are from normal control individuals. FL indicates full-length protein, and the arrowheads indicate the germ line truncated polypeptide. The arrows indicate molecular weight markers in kilodaltons (B) PCR analysis of DNA from a patient GC shows that the lesion in present in both hMLH3 alleles in tumor cells. Amplification was done using primers that amplify 5', 3', or within (MID) the region deleted in the cDNA. Lane 1, DNA derived from fibroblasts of patient GC; lane 2, DNA derived from tumor of patient GC; lane 3, DNA derived from a normal control patient; lane 4, reactions without DNA template. Arrows indicate molecular weight in base pairs.

In accordance with an aspect of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode for the mature polypeptides having the deduced amino acid sequence of Figures 1, 2 and 3 (SEQ ID No. 2, 4 and 6) or for the mature polypeptides encoded by the cDNA of the clone deposited as ATCC Deposit No. 75649, 75651, 75650, deposited on January 25, 1994.

ATCC Deposit No. 75649 is a cDNA clone which contains the full length sequence encoding the human DNA repair protein referred to herein as hMLH1; ATCC Deposit No. 75651 is a cDNA clone containing the full length cDNA sequence encoding the human DNA repair protein referred to herein as hMLH2; ATCC Deposit No. 75650 is a cDNA clone containing the full length DNA sequence referred to herein as hMLH3.

Polynucleotides encoding the polypeptides of the present invention may be obtained from one or more libraries prepared

from heart, lung, prostate, spleen, liver, gallbladder, fetal brain and testes tissues. The polynucleotides of hMLH1 were discovered from a human gallbladder cDNA library. In addition, six cDNA clones which are identical to the hMLH1 at the N-terminal ends were obtained from human cerebellum, eight-week embryo, fetal heart, HSC172 cells and Jurket cell cDNA libraries. The hMLH1 gene contains an open reading frame of 756 amino acids encoding for an 85kD protein which exhibits homology to the bacterial and yeast *mutL* proteins. However, the 5' non-translated region was obtained from the cDNA clone obtained from the fetal heart for the purpose of extending the non-translated region to design the oligonucleotides.

The hMLH2 gene was derived from a human T-cell lymphoma cDNA library. The hMLH2 cDNA clone identified an open reading frame of 2,796 base pairs flanked on both sides by in-frame termination codons. It is structurally related to the yeast PMS1 family. It contains an open reading frame encoding a protein of 934 amino acid residues. The protein exhibits the highest degree of homology to yeast PMS1 with 27% identity and 82 % similarity over the entire protein.

A second region of significant homology among the three PMS related proteins is in the carboxyl terminus, between codons 800 to 900. This region shares a 22% and 47% homology between yeast PMS1 protein and hMLH2 and hMLH3 proteins, respectively, while very little homology of this region was observed between these proteins, and the other yeast *mutL* homolog, yMLH1.

The hMLH3 gene was derived from a human endometrial tumor cDNA library. The hMLH3 clone identified a 2,586 base pair open reading frame. It is structurally related to the yPMS2 protein family. It contains an open reading frame encoding a protein of 862 amino acid residues. The protein exhibits the highest degree of homology to yPMS2 with 32%

identity and 66% similarity over the entire amino acid sequence.

It is significant with respect to a putative identification of hMLH1, hMLH2 and hMLH3 that the GFRGEAL domain which is conserved in *mutL* homologs derived from *E. coli* is conserved in the amino acid sequences of , hMLH1, hMLH2 and hMLH3.

The polynucleotides of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be identical to the coding sequence shown in Figures 1, 2 and 3 (SEQ ID No. 1) or that of the deposited clone or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptides as the DNA of Figures 1, 2 and 3 (SEQ ID No. 2, 4 and 6) or the deposited cDNA(s).

The polynucleotides which encode for the mature polypeptides of Figures 1, 2 and 3 (SEQ ID No. 2, 4 and 6) or for the mature polypeptides encoded by the deposited cDNAs may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptides having the deduced amino acid sequences of Figures 1, 2 and 3 (SEQ

ID No. 2, 4 and 6) or the polypeptides encoded by the cDNA of the deposited clones. The variants of the polynucleotides may be a naturally occurring allelic variant of the polynucleotides or a non-naturally occurring variant of the polynucleotides.

Thus, the present invention includes polynucleotides encoding the same mature polypeptides as shown in Figures 1, 2 and 3 (SEQ ID No. 2, 4 and 6) or the same mature polypeptides encoded by the cDNA of the deposited clones as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptides of Figures 1, 2 and 3 (SEQ ID No. 2, 4 and 6) or the polypeptides encoded by the cDNA of the deposited clones. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figures 1, 2 and 3 (SEQ ID No. 1, 3 and 5) or of the coding sequence of the deposited clones. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptides of the present invention. The marker sequence may be, for example, a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza

hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 50% and preferably 70% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which retain substantially the same biological function or activity as the mature polypeptides encoded by the cDNA of Figures 1, 2 and 3 (SEQ ID No. 1, 3 and 5) or the deposited cDNA(s).

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to polypeptides which have the deduced amino acid sequence of Figures 1, 2 and 3 (SEQ ID No. 2, 4 and 6) or which have the amino acid sequence encoded by the deposited cDNA(s), as well as fragments, analogs and derivatives of such polypeptides.

The terms "fragment," "derivative" and "analog" when referring to the polypeptides of Figures 1, 2 and 3 (SEQ ID No. 2, 4 and 6) or that encoded by the deposited cDNA(s), means polypeptides which retain essentially the same biological function or activity as such polypeptides. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptides of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptides of Figures 1, 2 and 3 (SEQ ID No. 2, 4 and 6) or that encoded by the deposited cDNAs may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol). Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the co-existing materials in the natural system, is isolated. Such

polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the hMLH1, hMLH2 and hMLH3 genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA

sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the proteins.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the

sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen, Inc.), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and TRP. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L.,

Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The

heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques

from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated.

In accordance with a further aspect of the invention, there is provided a process for determining susceptibility to cancer, in particular, a hereditary cancer. Thus, a mutation in a human repair protein, which is a human homolog of *mutL*, and in particular those described herein, indicates a susceptibility to cancer, and the nucleic acid sequences encoding such human homologs may be employed in an assay for ascertaining such susceptibility. Thus, for example, the assay may be employed to determine a mutation in a human DNA repair protein as herein described, such as a deletion, truncation, insertion, frame shift, etc., with such mutation being indicative of a susceptibility to cancer.

A mutation may be ascertained for example, by a DNA sequencing assay. Tissue samples, including but not limited to blood samples are obtained from a human patient. The samples are processed by methods known in the art to capture the RNA. First strand cDNA is synthesized from the RNA samples by adding an oligonucleotide primer consisting of polythymidine residues which hybridize to the polyadenosine stretch present on the mRNA's. Reverse transcriptase and deoxynucleotides are added to allow synthesis of the first strand cDNA. Primer sequences are synthesized based on the DNA sequence of the DNA repair protein of the invention. The primer sequence is generally comprised of 15 to 30 and preferably from 18 to 25 consecutive bases of the human DNA repair gene. Table 1 sets forth an illustrative example of oligonucleotide primer sequences based on hMLH1. The primers are used in pairs (one "sense" strand and one "anti-sense") to amplify the cDNA from the patients by the PCR method (Saiki *et al.*, Nature, 324:163-166 (1986)) such that three

overlapping fragments of the patient's cDNA's for such protein are generated. Table 1 also shows a list of preferred primer sequence pairs. The overlapping fragments are then subjected to dideoxynucleotide sequencing using a set of primer sequences synthesized to correspond to the base pairs of the cDNA's at a point approximately every 200 base pairs throughout the gene.

TABLE 1

Primer Sequences used to amplify gene region using PCR

Name	Start Site and Arrangement	Sequence
758	sense-(-41)*	GTTGAACATCTAGACGTCTC
1319	sense-8	TCGTGGCAGGGGTTATTCG
1321	sense-619	CTACCCAATGCCTCAACCG
1322	sense-677	GAGAACTGATAGAAATTGGATG
1314	sense-1548	GGGACATGAGGTTCTCCG
1323	sense-1593	GGGCTGTGTGAATCCTCAG
773	anti-53	CGGTTCACCACTGTCTCGTC
1313	anti-971	TCCAGGATGCTCTCCTCG
1320	anti-1057	CAAGTCCTGGTAGCAAAGTC
1315	anti-1760	ATGGCAAGGTCAAAGAGCG
1316	anti-1837	CAACAATGTATTCAGXAAGTCC
1317	anti-2340	TTGATACAACACTTTGTATCG
1318	anti-2415	GGAATACTATCAGAAGGCAAG

* Numbers corresponding to location along nucleotide sequence of Figure 1 where ATG is number 1.
Preferred primer sequences pairs:

758, 1313
1319, 1320
660, 1909
725, 1995
1680, 2536
1727, 2610

The nucleotide sequences shown in Table 1 represent SEQ ID No. 7 through 19, respectively.

Table 2 lists representative examples of oligonucleotide primer sequences (sense and anti-sense) which may be used, and preferably the entire set of primer sequences are used for sequencing to determine where a mutation in the patient DNA repair protein may be. The primer sequences may be from 15 to 30 bases in length and are preferably between 18 and 25 bases in length. The sequence information determined from the patient is then compared to non-mutated sequences to determine if any mutations are present.

TABLE 2

Primer Sequences Used to Sequence the Amplified Fragments

Name	Start Site Number	and Arrangement	Sequence
5282	seq01	sense-377*	ACAGAGCAAGTTACTCAGATG
5283	seq02	sense-552	GTACACAATGCAGGCATTAG
5284	seq03	sense-904	AATGTGGATGTTAATGTGCAC
5285	seq04	sense-1096	CTGACCTCGTCTTCCTAC
5286	seq05	sense-1276	CAGCAAGATGAGGAGATGC
5287	seq06	sense-1437	GGAAATGGTGGAAGATGATTC
5288	seq07	sense-1645	CTTCTCAACACCAAGC
5289	seq08	sense-1895	GAAATTGATGAGGAAGGGAAC
5295	seq09	sense-1921	CTTCTGATTGACAACTATGTGC
5294	seq10	sense-2202	CACAGAAGATGGAAATATCCTG
5293	seq11	sense-2370	GTGTTGGTAGCACTTAAGAC
5291	seq12	anti-525	TTTCCCATATTCTTCACTTG
5290	seq13	anti-341	GTAACATGAGCCACATGGC
5292	seq14	anti-46	CCACTGTCTCGTCCAGCCG

* Numbers corresponding to location along nucleotide sequence of Figure 1 where ATG is number 1.

The nucleotide sequences shown in Table 2 represent SEQ ID No. 20 through 33, respectively.

In another embodiment, the primer sequences from Table

2 could be used in the PCR method to amplify a mutated region. The region could be sequenced and used as a diagnostic to predict a predisposition to such mutated genes.

Alternatively, the assay to detect mutations in the genes of the present invention may be performed by genetic testing based on DNA sequence differences achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers *et al.*, Science, 230:1242 (1985)).

Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton *et al.*, PNAS, USA, 85:4397-4401 (1985)). Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, Western Blot analysis,

direct DNA sequencing or the use of restriction enzymes, (e.g., Restriction Fragment Length Polymorphisms (RFLP)) and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

The polypeptides may also be employed to treat cancers or to prevent cancers, by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present

invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells in vivo after combination with a suitable delivery vehicle.

Each of the cDNA sequences identified herein or a portion thereof can be used in numerous ways as polynucleotide reagents. The sequences can be used as diagnostic probes for the presence of a specific mRNA in a particular cell type. In addition, these sequences can be used as diagnostic probes suitable for use in genetic linkage analysis (polymorphisms).

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region is used to rapidly select primers that do not span more than one exon

in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome-specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than that have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clones from which the express sequence tag or EST was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than

4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

hMLH2 has been localized using a genomic P1 clone (1670) which contained the 5' region of the hMLH2 gene.

Detailed analysis of human metaphase chromosome spreads, counterstained to reveal banding, indicated that the hMLH2 gene was located within bands 2q32. Likewise, hMLH3 was localized using a genomic P1 clone (2053) which contained the 3' region of the hMLH3 gene. Detailed analysis of human metaphase chromosome spreads, counterstained to reveal banding, indicated that the hMLH3 gene was located within band 7p22, the most distal band on chromosome 7. Analysis with a variety of genomic clones showed that hMLH3 was a member of a subfamily of related genes, all on chromosome 7.

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can

be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Example 1

Bacterial Expression of hMLH1

The full length DNA sequence encoding human DNA mismatch repair protein hMLH1, ATCC # 75649, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence to synthesize

insertion fragments. The 5' oligonucleotide primer has the sequence 5' CGGGATCCATGTCGTTCGTGGCAGGG 3' (SEQ ID No. 34), contains a BamHI restriction enzyme site followed by 18 nucleotides of hMLH1 coding sequence following the initiation codon; the 3' sequence 5' GCTCTAGATTAACACCTCT CAAAGAC 3' (SEQ ID No. 35) contains complementary sequences to an XbaI site and is at the end of the gene. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). The plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His) and restriction enzyme cloning sites. The pQE-9 vector is digested with BamHI and XbaI and the insertion fragments are then ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the *E. coli* strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25

ug/ml). Tho O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized hMLH1 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., Genetic Engineering, Principles & Methods, 12:87-98 (1990). Protein renaturation out of GnHCl can be accomplished by several protocols (Jaenicke, R. and Rudolph, R., Protein Structure - A Practical Approach, IRL Press, New York (1990)). Initially, step dialysis is utilized to remove the GnHCL. Alternatively, the purified protein isolated from the Ni-chelate column can be bound to a second column over which a decreasing linear GnHCL gradient is run. The protein is allowed to renature while bound to the column and is subsequently eluted with a buffer containing 250 mM Imidazole, 150 mM NaCl, 25 mM Tris-HCl pH 7.5 and 10% Glycerol. Finally, soluble protein is dialyzed against a

storage buffer containing 5 mM Ammonium Bicarbonate. The purified protein was analyzed by SDS-PAGE.

Example 2

Spontaneous Mutation Assay for Detection of the Expression of hMLH1, hMLH2 and hMLH3 and Complementation to the *E.coli mutl*

The pQE9hMLH1, pQE9hMLH2 or pQE9hMLH3/GW3733, transformants were subjected to the spontaneous mutation assay. The plasmid vector pQE9 was also transformed to AB1157 (k-12, *argE3 hisG4,LeuB6 proA2 thr-1 ara-1 rpsL31 supE44 tsx-33*) and GW3733 to use as the positive and negative control respectively.

Fifteen 2 ml cultures, inoculated with approximately 100 to 1000 *E. coli*, were grown $2x10^8$ cells per ml in LB ampicillin medium at 37°C. Ten microliters of each culture were diluted and plated on the LB ampicillin plates to measure the number of viable cells. The rest of the cells from each culture were then concentrated in saline and plated on minimal plates lacking of arginine to measure reversion of Arg^+. In Table 3, the mean number of mutations per culture (m) was calculated from the median number (r) of mutants per distribution, according to the equation $(r/m)-\ln(m) = 1.24$ (Lea et al., J. Genetics 49:264-285 (1949)). Mutation rates per generation were recorded as m/N, with N representing the average number of cells per culture.

TABLE 3

Spontaneous Mutation Rates

Strain	Mutation/generation
AB1157+vector	(5.6±0.1) x 10-9a
GW3733+vector	(1.1±0.2) x 10-6a
GW3733+phMLH1	(3.7±1.3 x 10-7a
GW3733+phMLH2	(3.1±0.6) x 10-7b
GW3733+phMLH3	(2.1±0.8) x 10-7b

a: Average of three experiments.

b: Average of four experiments.

The functional complementation result showed that the human *mutL* can partially rescue the E.coli *mutL* mutator phenotype, suggesting that the human *mutL* is not only successfully expressed in a bacterial expression system, but also functions in bacteria.

Example 3

Chromosomal Mapping of the hMLH1

An oligonucleotide primer set was designed according to the sequence at the 5′ end of the cDNA for HMLH1. This primer set would span a 94 bp segment. This primer set was used in a polymerase chain reaction under the following set of conditions :

30 seconds, 95 degrees C

1 minute, 56 degrees C

1 minute, 70 degrees C

This cycle was repeated 32 times followed by one 5 minute cycle at 70 degrees C. Human, mouse, and hamster DNA were used as template in addition to a somatic cell hybrid panel (Bios, Inc). The reactions were analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. A 94 base pair band was observed in the human genomic DNA sample and in the somatic cell hybrid sample corresponding to chromosome 3. In addition, using various other somatic cell hybrid genomic DNA, the hMLH1 gene was localized to chromosome 3p.

Example 4

Method for Determination of mutation of hMLH1 gene in HNPCC kindred

cDNA was produced from RNA obtained from tissue samples from persons who are HNPCC kindred and the cDNA was used as a template for PCR, employing the primers 5' GCATC TAGACGTTTCCTTGGC 3' (SEQ ID No. 36) and 5' CATCCAAGCTTCTGT TCCCG 3' (SEQ ID No. 37), allowing amplification of codons 1 to 394 of Figure 1; 5' GGGGTGCAGCAGCACATCG 3' (SEQ ID No. 38) and 5' GGAGGCAGAATGTGTGAGCG 3' (SEQ ID No. 39), allowing amplification of codons 326 to 729 of Figure 1 (SEQ ID No. 2); and 5' TCCCAAAGAAGGACTTGCT 3' (SEQ ID No. 40) and 5' AGTATAAGTCTTAAGTGCTACC 3' (SEQ ID No. 41), allowing amplification of codons 602 to 756 plus 128 nt of

3'- untranslated sequences of Figure 1 (SEQ ID No. 2). The PCR conditions for all analyses used consisted of 35 cycles at 95°C for 30 seconds, 52-58°C for 60 to 120 seconds, and 70°C for 60 to 120 seconds, in the buffer solution described in San Sidransky, D. *et al.*, Science, 252:706 (1991). PCR products were sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase (Epicentre Technologies). The intron-exon borders of selected exons were also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations were then cloned and sequenced to validate the results of the direct sequencing. PCR products were cloned into T-tailed vectors as described in Holton, T.A. and Graham, M.W., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals from seven kindreds all exhibited a heterozygous deletion of codons 578 to 632 of the hMLH1 gene. The derivation of five of these seven kindreds could be traced to a common ancestor. The genomic sequences surrounding codons 578-632 were determined by cycle-sequencing of the P1 clones (a human genomic P1 library which contains the entire hMLH1 gene (Genome Systems)) using SequiTherm Polymerase, as described by the manufacturer, with the primers were labeled with T4 polynucleotide kinase, and by sequencing PCR products of genomic DNA. The primers used to amplify the exon

containing codons 578-632 were 5' TTTATGGTTTCTCACCTGCC 3' (SEQ ID No. 42) and 5' GTTATCTGCCCACCTCAGC 3' (SEQ ID No. 43). The PCR product included 105 bp of intron C sequence upstream of the exon and 117 bp downstream. No mutations in the PCR product were observed in the kindreds, so the deletion in the RNA was not due to a simple splice site mutation. Codons 578 to 632 were found to constitute a single exon which was deleted from the gene product in the kindreds described above. This exon contains several highly conserved amino acids.

In a second family (L7), PCR was performed using the above primers and a 4bp deletion was observed beginning at the first nucleotide (nt) of codon 727. This produced a frame shift with a new stop codon 166 nt downstream, resulting in a substitution of the carboxy-terminal 29 amino acids of hMLH1 with 53 different amino acids, some encoded by nt normally in the 3' untranslated region.

A different mutation was found in a different kindred (L2516) after PCR using the above primers, the mutation consisting of a 4bp insert between codons 755 and 756. This insertion resulted in a frame shift and extension of the ORF to include 102 nucleotides (34 amino acids) downstream of the normal termination codon. The mutations in both kindreds L7 and L2516 were therefore predicted to alter the C-terminus of hMLH1.

A possible mutation in the hMLH1 gene was determined from alterations in size of the encoded protein, where

kindreds were too few for linkage studies. The primers used for coupled transcription-translation of hMLH1 were 5' GGATCCTAATACGACTCACTATAGGGAGACCACCATGGCATCT AGACGTTTCCCTTGGC 3' (SEQ ID No. 44) and 5' CATCCAAGCTTCTGTTCCCG 3' (SEQ ID No. 45) for codons 1 to 394 of Figure 1 and 5' GGATCCTAATACGACTCACTATAGGGAGACCACCATGGG GGTGCAGCAGCACATCG 3' (SEQ ID No. 46) and 5' GGAGGCAGAATGTG TGAGCG 3' (SEQ ID No. 47) for codons 326 to 729 of Figure 1 (SEQ ID No. 2). The resultant PCR products had signals for transcription by T7 RNA polymerase and for the initiation of translation at their 5' ends. RNA from lymphoblastoid cells of patients from 18 kindreds was used to amplify two products, extending from codon 1 to codon 394 or from codon 326 to codon 729, respectively. The PCR products were then transcribed and translated *in vitro*, making use of transcription-translation signals incorporated into the PCR primers. PCR products were used as templates in coupled transcription-translation reactions performed as described by Powell, S.M. *et al.*, New England Journal of Medicine, 329:1982, (1993), using 40 micro CI of ^{35}S labeled methionine. Samples were diluted in sample buffer, boiled for five minutes and analyzed by electropheresis on sodium dodecyl sulfate-polyacrylamide gels containing a gradient of 10% to 20% acrylamide. The gels were dried and subjected to radiography. All samples exhibited a polypeptide of the expected size, but an abnormally migrating polypeptide was additionally found in one case.

The sequence of the relevant PCR product was determined and found to include a 371 bp deletion beginning at the first nucleotide (nt) of codon 347. This alteration was present in heterozygous form, and resulted in a frame shift in a new stop codon 30 nt downstream of codon 346, thus explaining the truncated polypeptide observed.

Four colorectal tumor cell lines manifesting microsatellite instability were examined. One of the four (cell line H6) showed no normal peptide in this assay and produced only a short product migrating at 27 kd. The sequence of the corresponding cDNA was determined and found to harbor a C to A transversion at codon 252, resulting in the substitution of a termination codon for serine. In accord with the translational analyses, no band at the normal C position was identified in the cDNA or genomic DNA from this tumor, indicating that it was devoid of a functional hMLH1 gene.

Table 4 sets forth the results of these sequencing assays. Deletions were found in those people who were known to have a family history of the colorectal cancer. More particularly, 9 of 10 families showed an hMLH1 mutation.

Table 4 - Summary of Mutations in *hMLH1*

Sample	Codon	cDNA Nucleotide Change	Predicted Coding Change
Kindreds F2, F3, F6, F8, F10, F11, F52	578-632	165 bp deletion	In-frame deletion
Kindred L7	727/728	4 bp deletion (TCACACATTC to TCATTCT)	Frameshift and substitution of new amino acids
Kindred L2516	755/756	4 bp insertion (GTGTTAA to GTGTTTGTTAA)	Extension of C-terminus
Kindred RA	347	371 bp deletion	Frameshift/ Truncation
H6 Colorectal Tumor	252	Transversion (TCA to TAA)	Serine to Stop

Example 5

Bacterial Expression and Purification of hMLH2

The DNA sequence encoding hMLH2, ATCC #75651, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence to synthesize insertion fragments. The 5' oligonucleotide primer has the sequence 5' CGGGATCCATGAAACAATTGCCTGCGGC 3' (SEQ ID No. 48) contains a BamHI restriction enzyme site

followed by 17 nucleotides of hMLH2 following the initiation codon. The 3' sequence 5' GCTCTAGACCAGACTCAT GCTGTTTT 3' (SEQ ID No. 49) contains complementary sequences to an XbaI site and is followed by 18 nucleotides of hMLH2. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. Chatsworth, CA). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. The amplified sequences and pQE-9 are then digested with BamHI and XbaI. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). Tho O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D._{600}$) of between 0.4 and 0.6.

IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized hMLH2 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., Genetic Engineering, Principles & Methods, 12:87-98 (1990). Protein renaturation out of GnHCl can be accomplished by several protocols (Jaenicke, R. and Rudolph, R., Protein Structure - A Practical Approach, IRL Press, New York (1990)). Initially, step dialysis is utilized to remove the GnHCL. Alternatively, the purified protein isolated from the Ni-chelate column can be bound to a second column over which a decreasing linear GnHCL gradient is run. The protein is allowed to renature while bound to the column and is subsequently eluted with a buffer containing 250 mM Imidazole, 150 mM NaCl, 25 mM Tris-HCl pH 7.5 and 10% Glycerol. Finally, soluble protein is dialyzed against a storage buffer containing 5 mM Ammonium Bicarbonate. The purified protein was analyzed by SDS-PAGE.

Example 6

Bacterial Expression and Purification of hMLH3

The DNA sequence encoding hMLH3, ATCC #75650, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence to synthesize insertion fragments. The 5' oligonucleotide primer has the sequence 5' CGGGATCCATGGAGCGAGCTGAGAGC 3' (SEQ ID No. 50) contains a BamHI restriction enzyme site followed by 18 nucleotides of hMLH3 coding sequence starting from the presumed terminal amino acid of the processed protein. The 3' sequence 5' GCTCTAGAGTGAAG ACTCTGTCT 3' (SEQ ID No. 51) contains complementary sequences to an XbaI site and is followed by 18 nucleotides of hMLH3. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. Chatsworth, CA). pQE-9 encodes antibiotic resistance (Amp'), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. The amplified sequences and pQE-9 are then digested with BamHI and XbaI. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture was then used to transform E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan').

Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). Tho O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D._{600}$) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized stanniocalcin is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., Genetic Engineering, Principles & Methods, 12:87-98 (1990). Protein renaturation out of GnHCl can be accomplished by several protocols (Jaenicke, R. and Rudolph, R., Protein Structure - A Practical Approach, IRL Press, New York (1990)). Initially, step dialysis is utilized to remove the GnHCL. Alternatively, the purified protein isolated from the Ni-chelate column can be bound to a second column

over which a decreasing linear GnHCL gradient is run. The protein is allowed to renature while bound to the column and is subsequently eluted with a buffer containing 250 mM Imidazole, 150 mM NaCl, 25 mM Tris-HCl pH 7.5 and 10% Glycerol. Finally, soluble protein is dialyzed against a storage buffer containing 5 mM Ammonium Bicarbonate. The purified protein was analyzed by SDS-PAGE.

Example 7

Method for determination of mutation of hMLH2 and hMLH3 in hereditary cancer

Isolation of Genomic Clones

A human genomic P1 library (Genomic Systems, Inc.) was screened by PCR using primers selected for the cDNA sequence of hMLH2 and hMLH3. Two clones were isolated for hMLH2 using primers 5' AAGCTGCTCTGTTAAAAGCG 3' (SEQ ID No. 52) and 5' GCACCAGCATCCAAGGAG 3' (SEQ ID No. 53) and resulting in a 133 bp product. Three clones were isolated for hMLH3, using primers 5' CAACCATGAGACACATCGC 3' (SEQ ID No. 54) and 5' AGGTTAGTGAAGACTCTGTC 3' (SEQ ID No. 55) resulting in a 121 bp product. Genomic clones were nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Manheim), and FISH was performed as described (Johnson, Cg. et al., Methods Cell Biol., 35:73-99 (1991)). Hybridization with the hMLH3 probe were carried out using a vast excess of human cot-1 DNA for specific hybridization to the expressed hMLH3 locus. Chromosomes were counterstained with 4,6-diamino-2-phenylidole andpropidium

iodide, producing a combination of C- and R-bands. Aligned images for precise mapping were obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters (Johnson, Cv. et al., Genet. Anal. Tech. Appl., 8:75 (1991)). Image collection, analysis and chromosomal fractional length measurements were done suing the ISee Graphical Program System (Inovision Corporation, Durham, NC).

Transcription coupled Translation Mutation Analysis

For purposes of IVSP analysis the hMLH2 gene was divided into three overlapping segments. The first segment included codons 1 to 500, while the middle segment included codons 270 to 755, and the last segment included codons 485 to the translational termination site at codon 933. The primers for the first segment were 5' GGATCCTAATACGACTCACT ATAGGGAGACCACCATGGAACAATTGCCTGCGG 3' (SEQ ID No. 56) and 5' CCTGCTCCACTCATCTGC 3' (SEQ ID No. 57), for the middle segment were 5' GGATCCTAATACGACTCACTATAGGGAGACCACCATGGAAGA TATCTTAAAGTTAATCCG 3' (SEQ ID No. 58) and 5' GGCTTCTTCTACTC TATATGG 3' (SEQ ID No. 59), and for the final segment were 5' GGATCCTAATACGACTCACTATAGGGAGACCACCATGGCAGGTCTTGAAAACTC TTCG 3' (SEQ ID No. 60) and 5' AAAACAAGTCAGTGAATCCTC 3' (SEQ ID No. 61). The primers used for mapping the stop mutation in patient CW all used the same 5' primer as the

first segment. The 3' nested primers were: 5' AAGCACATCTGTTTCTGCTG 3' (SEQ ID No. 62) codons 1 to 369; 5' ACGAGTAGATTCCTTTAGGC 3' (SEQ ID No. 63) codons 1 to 290; and 5' CAGAACTGACATGAGAGCC 3' (SEQ ID No. 64) codons 1 to 214.

For analysis of hMLH3, the hMLH3 cDNA was amplified as a full-length product or as two overlapping segments. The primers for full-length hMLH3 were 5' GGATCCTAATACGACTCACTATAGGGAGACCACCATGGAGCGAGCTGAGAGC 3' (SEQ ID No. 65) and 5' AGGTTAGTGAAGACTCTGTC 3' (SEQ ID No. 66) (codons 1 to 863). For segment 1, the sense primer was the same as above and the antisense primer was 5' CTGAGGTCT CAGCAGGC 3' (SEQ ID No. 67) (codons 1 to 472). Segment 2 primers were 5' GGATCCTAATACGACTCACTATAGGGAGACCACCATGGTGTC CATTTCCAGACTGCG 3' (SEQ ID No. 68) and 5' AGGTTAGTGAAGACTCT GTC 3' (SEQ ID No. 69) (codons 415 to 863). Amplifications were done as described below.

The PCR products contained recognition signals for transcription by T7 RNA polymerase and for the initiation of translation at thei 5' ends. PCR products were used as templates in coupled transcription-translation reactions containing 40 uCi of ^{36}S-methionine (NEN, Dupont). Samples were diluted in SDS sample buffer, and analyzed by electrophoresis on SDS-polyacrylamide gels containing a gradient of 10 to 20% acrylamide. The gels were fixed, treated with EnHance (Dupont), dried and subjected to autoradiography.

RT-PCR and Direct Sequencing of PCR Products

cDNAs were generated from RNA of lymphoblastoid or tumor cells with Superscript II (Life Technologies). The cDNAs were then used as templates for PCR. The conditions for all amplifications were 35 cycles at 95°C for 30s, 52°C to 62°C for 60 to 120s, and 70°C for 60 to 120s, in buffer. The PCR products were directly sequenced and cloned into the T-tailed cloning vector PCR2000 (Invitrogen) and sequenced with T7 polymerase (United States Biochemical). For the direct sequencing of PCR products, PCR reactions were first phenolchloroform extracted and ethanol precipitated. Templates were directly sequenced using Sequitherm polymerase (Epicentre Technologies) and gamma-^{32}P labelled primers as described by the manufacturer.

Intron/Exon Boundaries and Genomic Analysis of Mutations

Intron/exon borders were determined by cycle-sequencing P1 clones using gamma-^{32}P end labelled primers and SequiTherm polymerase as described by the manufacturer. The primers used to amplify the hMLH2 exon containing codons 195 to 233 were 5' TTATTTGGCAGAAAAGCAGAG (SEQ ID No. 70) 3' and 5' TTAAAAGACTAACCTCTTGCC 3' (SEQ ID No. 71), which produced a 215 bp product. The product was cycle sequenced using the primer 5' CTGCTGTTATGAACAATATGG 3' (SEQ ID No. 72). The primers used to analyze the genomic deletion of hMLH3 in patient GC were: for the 5' region

amplification 5' CAGAAGCAGTTGCAAAGCC 3' (SEQ ID No. 73) and 5' AAACCGTACTCTTCACACAC 3' (SEQ ID No. 74) which produces a 74 bp product containing codons 233 to 257, primers 5' GAGGAAAAGCTTTTGTTGGC 3' (SEQ ID No. 75) and 5' CAGTGGCTGCTGACTGAC 3' (SEQ ID No. 76) which produce a 93 bp product containing the codons 347 to 377, and primers 5' TCCAGAACCAAGAAGGAGC 3' (SEQ ID No. 77) and 5' TGAGGTCTCAGCAGGC 3' (SEQ ID No. 78) which produce a 99 bp product containing the codons 439 to 472 of hMLH3.

TABLE 5

Summary of Mutations in HMLH2 and HMLH3

from patients affected with HNPCC

Sample	Codon	Nucleotides	cDNA Change	Genomic Change	Predicted Coding Change
HMLH2					
CW	233		Skipped Exon	CAG to TAG	GLN to Stop Codon
HMLH3					
MM, NS, TF	20		CGG to CAG	CGG to CAG	ARG to GLN
GC	268 to 669		1,203 bp Deletion	Deletion	In-frame deletion
GCx	268 to 669		1,203 bp Deletion	Deletion	Frameshift, trucation

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: HUMAN GENOME SCIENCES, INC.

(ii) TITLE OF INVENTION: Human DNA Mismatch Repair Proteins

(iii) NUMBER OF SEQUENCES: 78

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN, CECCHI, STEWART & OLSTEIN
(B) STREET: 6 BECKER FARM ROAD
(C) CITY: ROSELAND
(D) STATE: NEW JERSEY
(E) COUNTRY: USA
(F) ZIP: 07068

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 INCH DISKETTE
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WORD PERFECT 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US95/01035
(B) FILING DATE: 25 JAN 1995
(C) CLASSIFICATION: UNASSIGNED

(v) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/294,312
(B) FILING DATE: 23 AUG 1994
(C) CLASSIFICATION:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/210,143
(B) FILING DATE: 16 MARCH 1994
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/187,757
(B) FILING DATE: 27 JAN 1994
(C) CLASSIFICATION:

(vi) ATTORNEY/AGENT INFORMATION:
(A) NAME: FERRARO, GREGORY D.
(B) REGISTRATION NUMBER: 36,134
(C) REFERENCE/DOCKET NUMBER: 325800-303

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(A) TELEPHONE: 201-994-1700
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 2525 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GGCCAGCTAA TGCTATCAAA GAGATGATTG AGAACTGTTT AGATGCAAAA TCCACAAGTA    180
TTCAAGTGAT TGTTAAAGAG GGAGGCCTGA AGTTGATTCA GATCCAAGAC AATGGCACCG    240
GGATCAGGAA AGAAGATCTG GATATTGTAT GTGAAAGTGT CACTACTAGT AAACTGCAGT    300
CCTTTGAGGA TTTAGCCAGT ATTTCTATCT ATGGCTTTCG AGGTGAGGCT TTGGCCAGCA    360
TAAGCCATGT GGCTCATGTT ACTATTACAA CGAAAACAGC TGATGGAAAG TGTGCATACA    420
GAGCAAGTTA CTCAGATGGA AAACTGAAAG CCCCTCCTAA ACCATGTGCT GGCAATCAAG    480
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AAAATCCAAG TGAAGAATAT GGGAAAATTT TGGAAGTTGT TGGCAGGTAT TCAGTACACA    600
ATGCAGGCAT TAGTTTCTCA GTTAAAAAAC AAGGAGAGAC AGTAGCTGAT GTTAGGACAC    660
TACCCAATGC CTCAACCGTG GACAATATTC GCTCCGTCTT GGGAAATGCT GTTAGTCGAG    720
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CCAATGCAAA CTACTCAGTG AAGAAGTGCA TCTTCTTACT CTTCATCAAC CATCGTCTGG    840
TAGAATCAAC TTCCTTGAGA AAAGCCATAG AAACAGTGTA TGCAGCCTAT TTGCCAAAAA    900
ACACACACCC ATTCCTGTAC CTCAGTTTAG AAATCAGTCC CCAGAATGTG GATGTTAATG    960
TGAACCCCAC AAAGCATGAA GTTCACTTCC TGCACGAGGA GAGCATCCTG GAGCGGGTGC   1020
AGCAGCACAT CGAGAGCAAG CTCCTGGGCT CCAATTCCTC CAGGATGTAC TTCACCCAGA   1080
CTTTGCTACC AGGACTTGCT GGCCCCTCTG GGGAGATGGT TAAATCCACA ACAAGTCTCA   1140
CCTCGTCTTC TACTTCTGGA AGTAGTGATA AGGTCTATGC CCACCAGATG GTTCGTACAG   1200
ATTCCCGGGA ACAGAAGCTT GATGCATTTC TGCAGCCTCT GAGCAAACCC CTGTCCAGTC   1260
AGCCCCAGGC CATTGTCACA GAGGATAAGA CAGATATTTC TAGTGGCAGG GCTAGGCAGC   1320
AAGATGAGGA GATGCTTGAA CTCCCAGCCC CTGCTGAAGT GGCTGCCAAA AATCAGAGCT   1380
TGGAGGGGGA TACAACAAAG GGGACTTCAG AAATGTCAGA GAAGAGAGGA CCTACTTCCA   1440
GCAACCCCAG AAAGAGACAT CGGGAAGATT CTGATCTCCA AATCCTCGAA GATGATTCCC   1500
GAAAGGAAAT GACTGCAGCT TGTACCCCCC GGAGAAGGAT CATTAACCTC ACTAGTGTTT   1560
TGAGTCTCCA GGAAGAAATT AATGAGCAGG GACATGAGGT TCTCCGGGAG ATGTTGCATA   1620
ACCACTCCTT CGTGGGCTGT GTGAATCCTC AGTGGGCCTT GGCACAGCAT CAAACCAAGT   1680
TATACCTTCT CAACACCACC AAGCTTAGTG AAGAACTGTT CTACCAGATA CTCATTTATG   1740
ATTTTGCCAA TTTTGGTGTT CTCAGGTTAT CGGAGCCAGC ACCGCTCTTT GACCTTGCCA   1800
TGCTTCCCTT ACATAGTCCA GAGAGTGGCT GGACAGAGGA AGATGGTCCC AAAGAAGGAC   1860
TTGCTGAATA CATTGTTGAG TTTCTGAAGA AGAAGGCTGA GATGCTTGCA GACTATTTCT   1920
CTTTGGAAAT TGATGAGGAA GGGAACCTGA TTGGATTACC CCTTCTGATT GACAACTATG   1980
TGCCCCCTTT GGAGGGACTG CCTATCTTCA TTCTTCCACT AGCCACTGAG GTGAATTGGG   2040
ACGAAGAAAA GGAATGTTTT GAAAGCCTCA GTAAAGAATG CGCTATGTTC TATTCCATCC   2100
GGAAGCAGTA CATATCTGAG GAGTCGACCC TCTCAGGCCA GCAGAGTGAA GTGCCTGGCT   2160
CCATTCCAAA CTCCTGGAAG TGGACTGTGG AACACATTGT CTATAAAGCC TTGCGCTCAC   2220
ACATTCTGCC TCCTAAACAT TCCACAGAAG ATGGAAATAT CCTGCAGCTT GCTAACCTGC   2280
CTGATCTATA CAAAGTCTTT GAGAGGTGTT AAATATGGTT ATTTATGCAC TGTGGGATGT   2340
GTTCTTCTTT CTCTGTATTC CGATACAAAG TGTTGTACTA AAGTGTGATA TACAAAGTGT   2400
ACCAACATAA GTGTTGGTAG CACTTAAGAC TTATACTTGC CTTCTGATAG TATTCCTTTA   2460
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AAAAA                                                               2525
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(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 756 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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            20                  25                  30
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        35                  40                  45
Ser Ile Gln Val Ile Val Lys Glu Gly Gly Leu Lys Leu Ile Gln
    50                  55                  60
Ile Gln Asp Asn Gly Thr Gly Ile Arg Lys Glu Asp Leu Asp Ile
65                  70                  75
Val Cys Glu Arg Phe Thr Thr Ser Lys Leu Gln Ser Phe Glu Asp
                80                  85                  90
Leu Ala Ser Ile Ser Thr Tyr Gly Phe Arg Gly Glu Ala Leu Ala
            95                  100                 105
Ser Ile Ser His Val Ala His Val Thr Ile Thr Thr Lys Thr Ala
        110                 115                 120
Asp Gly Lys Cys Ala Tyr Arg Ala Ser Tyr Ser Asp Gly Lys Leu
    125                 130                 135
Lys Ala Pro Pro Lys Pro Cys Ala Gly Asn Gln Gly Thr Gln Ile
140                 145                 150
Thr Val Glu Asp Leu Phe Tyr Asn Ile Ala Thr Arg Arg Lys Ala
            155                 160                 165
Leu Lys Asn Pro Ser Glu Glu Tyr Gly Lys Ile Leu Glu Val Val
        170                 175                 180
Gly Arg Tyr Ser Val His Asn Ala Gly Ile Ser Phe Ser Val Lys
    185                 190                 195
Lys Gln Gly Glu Thr Val Ala Asp Val Arg Thr Leu Pro Asn Ala
200                 205                 210
Ser Thr Val Asp Asn Ile Arg Ser Val Phe Gly Asn Ala Val Ser
            215                 220                 225
Arg Glu Leu Ile Glu Ile Gly Cys Glu Asp Lys Thr Leu Ala Phe
        230                 235                 240
Lys Met Asn Gly Tyr Ile Ser Asn Ala Asn Tyr Ser Val Lys Lys
    245                 250                 255
Cys Ile Phe Leu Leu Phe Ile Asn His Arg Leu Val Glu Ser Thr
260                 265                 270
Ser Leu Arg Lys Ala Ile Glu Thr Val Tyr Ala Ala Tyr Leu Pro
            275                 280                 285
Lys Asn Thr His Pro Phe Leu Tyr Leu Ser Leu Glu Ile Ser Pro
        290                 295                 300
Gln Asn Val Asp Val Asn Val His Pro Thr Lys His Glu Val His
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365 370 375
Asp Lys Val Tyr Ala His Gln Met Val Arg Thr Asp Ser Arg Glu
380 385 390
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395 400 405
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425 430 435
Ala Pro Ala Glu Val Ala Ala Lys Asn Gln Ser Leu Glu Gly Asp
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455 460 465
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Met Val Glu Asp Asp Ser Arg Lys Glu Met Thr Ala Ala Cys Thr
485 490 495
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Glu Glu Ile Asn Glu Gln Gly His Glu Val Leu Arg Glu Met Leu
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Asp Gly Pro Lys Glu Gly Leu Ala Glu Tyr Ile Val Glu Phe Leu
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Ala Thr Glu Val Asn Trp Asp Glu Glu Lys Glu Cys Phe Glu Ser
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Ile Ser Glu Glu Ser Thr Leu Ser Gly Gln Gln Ser Glu Val Pro

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Tyr Lys Ala Leu Arg Ser His Ile Leu Pro Pro Lys His Phe Thr
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 3063 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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CTTCACATCT TGGTCAAGGT ACAACTGTAA CTGCTTTAAG ATTATTTAAG AATCTACCTG     540
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ATCTCCTCAT GAGCTTTGGT ATCCTTAAAC CTGACTTAAG GATTGTCTTT GTACATAACA     660
AGGCAGTTAT TTGGCAGAAA AGCAGAGTAT CAGATCACAA GATGGCTCTC ATGTCAGTTC     720
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AGTTAATCCG ACATCATTAC AATCTGAAAT GCCTAAAGGA ATCTACTCGT TTGTATCCTG     960
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AATCATCTGG AAAGAATTAT TCAAATGTTG ATACTTCAGT CATTCCATTC CAAAATGATA    1260
TGCATAATGA TGAATCTGGA AAAAACACTG ATGATTGTTT AAATCACCAG ATAAGTATTG    1320
GTGACTTTGG TTATGGTCAT TGTAGTAGTG AAATTTCTAA CATTGATAAA AACACTAAGA    1380
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GTAAAACTTG TTTTATAAGT TCCGTTAAGC ACACCCAGTC AGAAAATGGC AATAAAGACC    1500
ATATAGATGA GAGTGGGGAA AATGAGGAAG AAGCAGGTCT TGAAAACTCT TCGGAAATTT    1560
CTGCAGATGA GTGGAGCAGG GGAAATATAC TTAAAAATTC AGTGGGAGAG AATATTGAAC    1620
CTGTGAAAAT TTTAGTGCCT GAAAAAAGTT TACCATGTAA AGTAAGTAAT AATAATTATC    1680
CAATCCCTGA ACAAATGAAT CTTAATGAAG ATTCATGTAA CAAAAAATCA AATGTAATAG    1740
ATAATAAATC TGGAAAAGTT ACAGCTTATG ATTTACTTAG CAATCGAGTA ATCAAGAAAC    1800
CCATGTCAGC AAGTGCTCTT TTTGTTCAAG ATCATCGTCC TCAGTTTCTC ATAGAAAATC    1860
CTAAGACTAG TTTAGAGGAT GCAACACTAC AAATTGAAGA ACTGTGGAAG ACATTGAGTG    1920
AAGAGGAAAA ACTGAAATAT GAAGAGAAGG CTACTAAAGA CTTGGNACGA TACAATAGTC    1980
AAATGAAGAG AGCCATTGAA CAGGAGTCAC AAATGTCACT AAAAGATGGC AGAAAAAAGA    2040
TAAAACCCAC CAGCGCATGG AATTTGGCCC AGAAGCACAA GTTAAAAACC TCATTATCTA    2100
ATCAACCANA ACTTGATGAA CTCCTTCAGT CCCAAATTGA AAAAAGAAGG AGTCAAAATA    2160
TTAAAATGGT ACAGATCCCC TTTTCTATGA AAAACTTAAA AATAAATTTT AAGAAACAAA    2220
```

```
ACAAAGTTGA CTTAGAAGAG AAGGATGAAC CTTGCTTGAT CCACAATCTC AGGTTTCCTG    2280
ATGCATGGCT AATGACATCC AAAACAGAGG TAATGTTATT AAATCCATAT AGAGTAGAAG    2340
AAGCCCTGCT ATTTAAAAGA CTTCTTGAGA ATCATAAACT TCCTGCAGAG CCACTGGAAA    2400
AGCCAATTAT GTTAACAGAG AGTCTTTTTA ATGGATCTCA TTATTTAGAC GTTTTATATA    2460
AAATGACAGC AGATGACCAA AGATACAGTG GATCAACTTA CCTGTCTGAT CCTCGTCTTA    2520
CAGCGAATGG TTTCAAGATA AAATTGATAC CAGGAGTTTC AATTACTGAA AATTACTTGG    2580
AAATAGAAGG AATGGCTAAT TGTCTCCCAT TCTATGGAGT AGCAGATTTA AAAGAAATTC    2640
TTAATGCTAT ATTAAACAGA AATGCAAAGG AAGTTTATGA ATGTAGACCT CGCAAAGTGA    2700
TAAGTTATTT AGAGGGAGAA GCAGTGCGTC TATCCAGACA ATTACCCATG TACTTATCAA    2760
AAGAGGACAT CCAAGACATT ATCTACAGAA TGAAGCACCA GTTTGGAAAT GAAATTAAAG    2820
AGTGTGTTCA TGGTCGCCCA TTTTTTCATC ATTTAACCTA TCTTCCAGAA ACTACATGAT    2880
TAAATATGTT TAAGAAGATT AGTTACCATT GAAATTGGTT CTGTCATAAA ACAGCATGAG    2940
TCTGGTTTTA AATTATCTTT GTATTATGTG TCACATGGTT ATTTTTTAAA TGAGGATTCA    3000
CTGACTTGTT TTTATATTGA AAAAAGTTCC ACGTATTGTA GAAAACGTAA ATAAACTAAT    3060
AAC                                                                  3063
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 931 BASE PAIRS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN (XI)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Lys Gln Leu Pro Ala Ala Thr Val Arg Leu Leu Ser Ser Ser
 5                  10                  15
Gln Ile Ile Thr Ser Val Val Ser Val Val Lys Glu Leu Ile Glu
            20                  25                  30
Asn Ser Leu Asp Ala Gly Ala Thr Ser Val Asp Val Lys Leu Glu
        35                  40                  45
Asn Tyr Gly Phe Asp Lys Ile Glu Val Arg Asp Asn Gly Glu Gly
    50                  55                  60
Ile Lys Ala Val Asp Ala Pro Val Met Ala Met Lys Tyr Tyr Thr
65                  70                  75
Ser Lys Ile Asn Ser His Gly Asp Leu Glu Asn Leu Thr Thr Tyr
                80                  85                  90
Gly Phe Arg Gly Glu Ala Leu Gly Ser Ile Cys Cys Ile Ala Glu
            95                  100                 105
Val Leu Ile Thr Thr Arg Thr Ala Ala Asp Asn Phe Ser Thr Gln
        110                 115                 120
Tyr Val Leu Asp Gly Ser Gly His Ile Leu Ser Gln Lys Pro Ser
    125                 130                 135
His Leu Gly Gln Gly Thr Thr Val Thr Ala Leu Arg Leu Phe Lys
140                 145                 150
Asn Leu Pro Val Arg Lys Gln Phe Tyr Ser Thr Ala Lys Lys Cys
155                 160                 165
Lys Asp Glu Ile Lys Lys Ile Gln Asp Leu Leu Met Ser Phe Gly
            170                 175                 180
Ile Leu Lys Pro Asp Leu Arg Ile Val Phe Val His Asn Lys Ala
        185                 190                 195
Val Ile Trp Gln Lys Ser Arg Val Ser Asp His Lys Met Ala Leu
```

200 205 210
Met Ser Val Leu Gly Thr Ala Val Met Asn Asn Met Glu Ser Phe
215 220 225
Gln Tyr His Ser Glu Glu Ser Gln Ile Tyr Leu Ser Gly Phe Leu
230 235 240
Pro Lys Cys Asp Ala Asp His Ser Phe Thr Ser Leu Ser Thr Pro
245 250 255
Glu Arg Ser Phe Ile Phe Ile Asn Ser Arg Pro Val His Gln Lys
260 265 270
Asp Ile Leu Lys Leu Ile Arg His His Tyr Asn Leu Lys Cys Leu
275 280 285
Lys Glu Ser Thr Arg Leu Tyr Pro Val Phe Phe Leu Lys Ile Asp
290 295 300
Val Pro Thr Ala Asp Val Asp Val Asn Leu Thr Pro Asp Lys Ser
305 310 315
Gln Val Leu Leu Gln Asn Lys Glu Ser Val Leu Ile Ala Leu Glu
320 325 330
Asn Leu Met Thr Thr Cys Tyr Gly Pro Leu Pro Ser Thr Asn Ser
335 340 345
Tyr Glu Asn Asn Lys Thr Asp Val Ser Ala Ala Asp Ile Val Leu
350 355 360
Ser Lys Thr Ala Glu Thr Asp Val Leu Phe Asn Lys Val Glu Ser
365 370 375
Ser Gly Lys Asn Tyr Ser Asn Val Asp Thr Ser Val Ile Pro Phe
380 385 390
Gln Asn Asp Met His Asn Asp Glu Ser Gly Lys Asn Thr Asp Asp
395 400 405
Cys Leu Asn His Gln Ile Ser Ile Gly Asp Phe Gly Tyr Gly His
410 415 420
Cys Ser Ser Glu Ile Ser Asn Ile Asp Lys Asn Thr Lys Asn Ala
425 430 435
Phe Gln Asp Ile Ser Met Ser Asn Val Ser Trp Glu Asn Ser Gln
440 445 450
Thr Glu Tyr Ser Lys Thr Cys Phe Ile Ser Ser Val Lys His Thr
455 460 465
Gln Ser Glu Asn Gly Asn Lys Asp His Ile Asp Glu Ser Gly Glu
470 475 480
Asn Glu Glu Glu Ala Gly Leu Glu Asn Ser Ser Glu Ile Ser Ala
485 490 495
Asp Glu Trp Ser Arg Gly Asn Ile Leu Lys Asn Ser Val Gly Glu
500 505 510
Asn Ile Glu Pro Val Lys Ile Leu Val Pro Glu Lys Ser Leu Pro
515 520 525
Cys Lys Val Ser Asn Asn Asn Tyr Pro Ile Pro Glu Gln Met Asn
530 535 540
Leu Asn Glu Asp Ser Cys Asn Lys Lys Ser Asn Val Ile Asp Asn
545 550 555
Lys Ser Gly Lys Val Thr Ala Tyr Asp Leu Leu Ser Asn Arg Val
560 565 570
Ile Lys Lys Pro Met Ser Ala Ser Ala Leu Phe Val Gln Asp His
575 580 585
Arg Pro Gln Phe Leu Ile Glu Asn Pro Lys Thr Ser Leu Glu Asp

590 595 600
Ala Thr Leu Gln Ile Glu Glu Leu Trp Lys Thr Leu Ser Glu Glu
605 610 615
Glu Lys Leu Lys Tyr Glu Glu Lys Ala Thr Lys Asp Leu Xaa Arg
620 625 630
Tyr Asn Ser Gln Met Lys Arg Ala Ile Glu Gln Glu Ser Gln Met
635 640 645
Ser Leu Lys Asp Gly Arg Lys Lys Ile Lys Pro Thr Ser Ala Trp
650 655 660
Asn Leu Ala Gln Lys His Lys Leu Lys Thr Ser Leu Ser Asn Gln
665 670 675
Pro Xaa Leu Asp Glu Leu Leu Gln Ser Gln Ile Glu Lys Arg Arg
680 685 690
Ser Gln Asn Ile Lys Met Val Gln Ile Pro Phe Ser Met Lys Asn
695 700 705
Leu Lys Ile Asn Phe Lys Lys Gln Asn Lys Val Asp Leu Glu Glu
710 715 720
Lys Asp Glu Pro Cys Leu Ile His Asn Leu Arg Phe Pro Asp Ala
725 730 735
Trp Leu Met Thr Ser Lys Thr Glu Val Met Leu Leu Asn Pro Tyr
740 745 750
Arg Val Glu Glu Ala Leu Leu Phe Lys Arg Leu Leu Glu Asn His
755 760 765
Lys Leu Pro Ala Glu Pro Leu Glu Lys Pro Ile Met Leu Thr Glu
770 775 780
Ser Leu Phe Asn Gly Ser His Tyr Leu Asp Val Leu Tyr Lys Met
785 790 795
Thr Ala Asp Asp Gln Arg Tyr Ser Gly Ser Thr Tyr Leu Ser Asp
800 805 810
Pro Arg Leu Thr Ala Asn Gly Phe Lys Ile Lys Leu Ile Pro Gly
815 820 825
Val Ser Ile Thr Glu Asn Tyr Leu Glu Ile Glu Gly Met Ala Asn
830 835 840
Cys Leu Pro Phe Tyr Gly Val Ala Asp Leu Lys Glu Ile Leu Asn
845 850 855
Ala Ile Leu Asn Arg Asn Ala Lys Glu Val Tyr Glu Cys Arg Pro
860 865 870
Arg Lys Val Ile Ser Tyr Leu Glu Gly Glu Ala Val Arg Leu Ser
875 880 885
Arg Gln Leu Pro Met Tyr Leu Ser Lys Glu Asp Ile Gln Asp Ile
890 895 900
Ile Tyr Arg Met Lys His Gln Phe Gly Asn Glu Ile Lys Glu Cys
905 910 915
Val His Gly Arg Pro Phe Phe His His Leu Thr Tyr Leu Pro Glu
920 925 930
Thr

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 2771 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CGAGGCGGAT CGGGTGTTGC ATCCATGGAG CGAGCTGAGA GCTCGAGTAC AGAACCTGCT      60
AAGGCCATCA AACCTATTGA TCGGAAGTCA GTCCATCAGA TTTGCTCTGG GCAGGTGGTA     120
CTGAGTCTAA GCACTGCGGT AAAGGAGTTA GTAGAAAACA GTCTGGATGC TGGTGCCACT     180
AATATTGATC TAAAGCTTAA GGACTATGGA GTGGATCTTA TTGAAGTTTC AGACAATGGA     240
TGTGGGGTAG AAGAAGAAAA CTTCGAAGGC TTAACTCTGA AACATCACAC ATCTAAGATT     300
CAAGAGTTTG CCGACCTAAC TCAGGTTGAA ACTTTTGGCT TTCGGGGGGA AGCTCTGAGC     360
TCACTTTGTG CACTGAGCGA TGTCACCATT TCTACCTGCC ACGCATCGGC GAAGGTTGGA     420
ACTCGACTGA TGTTTGATCA CAATGGGAAA ATTATCCAGA AAACCCCCTA CCCCCGCCCC     480
AGAGGGACCA CAGTCAGCGT GCAGCAGTTA TTTTCCACAC TACCTGTGCG CCATAAGGAA     540
TTTCAAAGGA ATATTAAGAA GGAGTATGCC AAAATGGTCC AGGTCTTACA TGCATACTGT     600
ATCATTTCAG CAGGCATCCG TGTAAGTTGC ACCAATCAGC TTGGACAAGG AAAACGACAG     660
CCTGTGGTAT GCACAGGTGG AAGCCCCAGC ATAAAGGAAA ATATCGGCTC TGTGTTTGGG     720
CAGAAGCAGT TGCAAAGCCT CATTCCTTTT GTTCAGCTGC CCCCTAGTGA CTCCGTGTGT     780
GAAGAGTACG GTTTGAGCTG TTCGGATGCT CTGCATAATC TTTTTTACAT CTCAGGTTTC     840
ATTTCACAAT GCACGCATGG AGTTGGAAGG AGTTCAACAG ACAGACAGTT TTTCTTTATC     900
AACCGGCGGC CTTGTGACCC AGCAAAGGTC TGCAGACTCG TGAATGAGGT CTACCACATG     960
TATAATCGAC ACCAGTATCC ATTTGTTGTT CTTAACATTT CTGTTGATTC AGAATGCGTT    1020
GATATCAATG TTACTCCAGA TAAAAGGCAA ATTTTGCTAC AAGAGGAAAA GCTTTTGTTG    1080
GCAGTTTTAA AGACCTCTTT GATAGGAATG TTTGATAGTG ATGTCAACAA GCTAAATGTC    1140
AGTCAGCAGC CACTGCTGGA TGTTGAAGGT AACTTAATAA AAATGCATGC AGCGGATTTG    1200
GAAAAGCCCA TGGTAGAAAA GCAGGATCAA TCCCCTTCAT TAAGGACTGG AGAAGAAAAA    1260
AAAGACGTGT CCATTTCCAG ACTGCGAGAG GCCTTTTCTC TTCGTCACAC AACAGAGAAC    1320
AAGCCTCACA GCCCAAAGAC TCCAGAACCA AGAAGGAGCC CTCTAGGACA GAAAAGGGGT    1380
ATGCTGTCTT CTAGCACTTC AGGTGCCATC TCTGACAAAG GCGTCCTGAG ACCTCAGAAA    1440
GAGGCAGTGA GTTCCAGTCA CGGACCCAGT GACCCTACGG ACAGAGCGGA GGTGGAGAAG    1500
GACTCGGGGC ACGGCAGCAC TTCCGTGGAT TCTGAGGGGT TCAGCATCCC AGACACGGGC    1560
AGTCACTGCA GCAGCGAGTA TGCGGCCAGC TCCCCAGGGG ACAGGGGCTC GCAGGAACAT    1620
GTGGACTCTC AGGAGAAAGC GCCTGAAACT GACGACTCTT TTTCAGATGT GGACTGCCAT    1680
TCAAACCAGG AAGATACCGG ATGTAAATTT CGAGTTTTGC CTCAGCCAAC TAATCTCGCA    1740
ACCCCAAACA CAAAGCGTTT TAAAAAAGAA GAAATTCTTT CCAGTTCTGA CATTTGTCAA    1800
AAGTTAGTAA ATACTCAGGA CATGTCAGCC TCTCAGGTTG ATGTAGCTGT GAAAATTAAT    1860
AAGAAAGTTG TGCCCCTGGA CTTTTCTATG AGTTCTTTAG CTAAACGAAT AAAGCAGTTA    1920
CATCATGAAG CACAGCAAAG TGAAGGGGAA CAGAATTACA GGAAGTTTAG GGCAAAGATT    1980
TGTCCTGGAG AAAATCAAGC AGCCGAAGAT GAACTAAGAA AAGAGATAAG TAAAACGATG    2040
TTTGCAGAAA TGGAAATCAT TGGTCAGTTT AACCTGGGAT TTATAATAAC CACACTGAAT    2100
GAGGATATCT TCATAGTGGA CCAGCATGCC ACGGACGAGA AGTATAACTT CGAGATGCTG    2160
CAGCAGCACA CCGTGCTCCA GGGGCAGACG CTCATAGCAC CTCAGACTCT CAACTTAACT    2220
GCTGTTAATG AAGCTGTTCT GATAGAAAAT CTGGAAATAT TTAGAAAGAA TGGCTTTGAT    2280
TTTGTTATCG ATGAAAATGC TCCAGTCACT GAAAGGGCTA AACTGATTTC CTTGCCAACT    2340
AGTAAAAACT GGACCTTCGG ACCCCAGGAC GTCGATGAAC TGATCTTCAT GCTGAGCGAC    2400
AGCCCTGGGG TCATGTGCCG GCCTTCCCGA GTCAAGCAGA TGTTTGCCTC CAGAGCCTGC    2460
CGGAAGTCGG TGATGATTGG GACTGCTCTT AACACAAGCG AGATGAAGAA ACTGATCACC    2520
CACATGGGGG AGATGGACCA CCCCTGGAAC TGTCCCCATG GAAGGCCAAC CATGAGACAC    2580
ATCGCCAACC TGGGTGTCAT TTCTCAGAAC TGACCGTAGT CACTGTATGG AATAATTGGT    2640
TTTATCGCAG ATTTTTATGT TTTGAAAGAC AGAGTCTTCA CTAACCTTTT TTGTTTTAAA    2700
ATGAAACCTG CTACTTAAAA AAAATACACA TCACACCCAT TTAAAAGTGA TCTTGAGAAC    2760
CTTTTCAAAC C                                                        2771
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 862 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Glu Arg Ala Glu Ser Ser Ser Thr Glu Pro Ala Lys Ala Ile
5 10 15
Lys Pro Ile Asp Arg Lys Ser Val His Gln Ile Cys Ser Gly Gln
20 25 30
Val Val Leu Ser Leu Ser Thr Ala Val Lys Glu Leu Val Glu Asn
35 40 45
Ser Leu Asp Ala Gly Ala Thr Asn Ile Asp Leu Lys Leu Lys Asp
50 55 60
Tyr Gly Val Asp Leu Ile Glu Val Ser Asp Asn Gly Cys Gly Val
65 70 75
Glu Glu Glu Asn Phe Glu Gly Leu Thr Leu Lys His His Thr Ser
80 85 90
Lys Ile Gln Glu Phe Ala Asp Leu Thr Gln Val Glu Thr Phe Gly
95 100 105
Phe Arg Gly Glu Ala Leu Ser Ser Leu Cys Ala Leu Ser Asp Val
110 115 120
Thr Ile Ser Thr Cys His Ala Ser Ala Lys Val Gly Thr Arg Leu
125 130 135
Met Phe Asp His Asn Gly Lys Ile Ile Gln Lys Thr Pro Tyr Pro
140 145 150
Arg Pro Arg Gly Thr Thr Val Ser Val Gln Gln Leu Phe Ser Thr
155 160 165
Leu Pro Val Arg His Lys Glu Phe Gln Arg Asn Ile Lys Lys Glu
170 175 180
Tyr Ala Lys Met Val Gln Val Leu His Ala Tyr Cys Ile Ile Ser
185 190 195
Ala Gly Ile Arg Val Ser Cys Thr Asn Gln Leu Gly Gln Gly Lys
200 205 210
Arg Gln Leu Trp Tyr Ala Gln Val Glu Ala Pro Ala Ile Lys Glu
215 220 225
Asn Ile Gly Ser Val Phe Gly Gln Lys Gln Leu Gln Ser Leu Ile
230 235 240
Pro Phe Val Gln Leu Pro Pro Ser Asp Ser Val Cys Glu Glu Tyr
245 250 255
Gly Leu Ser Cys Ser Asp Ala Leu His Asn Leu Phe Tyr Ile Ser
260 265 270
Gly Phe Ile Ser Gln Cys Thr His Gly Val Gly Arg Ser Ser Thr
275 280 285
Asp Arg Gln Phe Phe Phe Ile Asn Arg Arg Pro Cys Asp Pro Ala
290 295 300
Lys Val Cys Arg Leu Val Asn Glu Val Tyr His Met Tyr Asn Arg
305 310 315
His Gln Tyr Pro Phe Val Val Leu Asn Ile Ser Val Asp Ser Glu
320 325 330
Cys Val Asp Ile Asn Val Thr Pro Asp Lys Arg Gln Ile Leu Leu
335 340 345

Gln Glu Glu Lys Leu Leu Leu Ala Val Leu Lys Thr Ser Leu Ile
350 355 360
Gly Met Phe Asp Ser Asp Val Asn Lys Leu Asn Val Ser Gln Gln
365 370 375
Pro Leu Leu Asp Val Glu Gly Asn Leu Ile Lys Met His Ala Ala
380 385 390
Asp Leu Glu Lys Pro Met Val Glu Lys Gln Asp Gln Ser Pro Ser
395 400 405
Leu Arg Thr Gly Glu Glu Lys Lys Asp Val Ser Ile Ser Arg Leu
410 415 420
Arg Glu Ala Phe Ser Leu Arg His Thr Thr Glu Asn Lys Pro His
425 430 435
Ser Pro Lys Thr Pro Glu Pro Arg Arg Ser Pro Leu Gly Gln Lys
440 445 450
Arg Gly Met Leu Ser Ser Ser Thr Ser Gly Ala Ile Ser Asp Lys
455 460 465
Gly Val Leu Arg Pro Gln Lys Glu Ala Val Ser Ser Ser His Gly
470 475 480
Pro Ser Asp Pro Thr Asp Arg Ala Glu Val Glu Lys Asp Ser Gly
485 490 495
His Gly Ser Thr Ser Val Asp Ser Glu Gly Phe Ser Ile Pro Asp
500 505 510
Thr Gly Ser His Cys Ser Ser Glu Tyr Ala Ala Ser Ser Pro Gly
515 520 525
Asp Arg Gly Ser Gln Glu His Val Asp Ser Gln Glu Lys Ala Pro
530 535 540
Glu Thr Asp Asp Ser Phe Ser Asp Val Asp Cys His Ser Asn Gln
545 550 555
Glu Asp Thr Gly Cys Lys Phe Arg Val Leu Pro Gln Pro Thr Asn
560 565 570
Leu Ala Thr Pro Asn Thr Lys Arg Phe Lys Lys Glu Glu Ile Leu
575 580 585
Ser Ser Ser Asp Ile Cys Pro Gln Leu Val Asn Thr Gln Asp Met
590 595 600
Ser Ala Ser Gln Val Asp Val Ala Val Lys Ile Asn Lys Lys Val
605 610 615
Val Pro Leu Asp Phe Ser Met Ser Ser Leu Ala Lys Arg Ile Lys
620 625 630
Gln Leu His His Glu Ala Gln Gln Ser Glu Gly Glu Gln Asn Tyr
635 640 645
Arg Lys Phe Arg Ala Lys Ile Cys Pro Gly Glu Asn Gln Ala Ala
650 655 660
Glu Asp Glu Leu Arg Lys Glu Ile Ser Lys Thr Met Phe Ala Glu
665 670 675
Met Glu Ile Ile Gly Gln Phe Asn Leu Gly Phe Ile Ile Thr Thr
680 685 690
Leu Asn Glu Asp Ile Phe Ile Val Asp Glu His Ala Thr Asp Glu
695 700 705
Lys Tyr Asn Phe Glu Met Leu Gln Gln His Thr Val Leu Gln Gly
710 715 720
Gln Arg Leu Ile Ala Pro Glu Thr Leu Asn Leu Thr Ala Val Asn
725 730 735

Glu Ala Val Leu Ile Glu Asn Leu Glu Ile Phe Arg Lys Asn Gly
740 745 750
Phe Asp Phe Val Ile Asp Glu Asn Ala Pro Val Thr Glu Arg Ala
755 760 765
Lys Leu Ile Ser Leu Pro Thr Ser Lys Asn Trp Thr Phe Gly Pro
770 775 780
Gln Asp Val Asp Glu Leu Ile Phe Met Leu Ser Asp Ser Pro Gly
785 790 795
Val Met Cys Arg Pro Ser Arg Val Lys Gln Met Phe Ala Ser Arg
800 805 810
Ala Cys Arg Lys Ser Val Met Ile Gly Thr Ala Leu Asn Thr Ser
815 820 825
Glu Met Lys Lys Leu Ile Thr His Met Gly Glu Met Asp His Pro
830 835 840
Trp Asn Cys Pro His Gly Arg Pro Thr Met Arg His Ile Ala Asn
845 850 855
Leu Gly Val Ile Ser Gln Asn
860

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GTTGAACATC TAGACGTCTC 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TCGTGGCAGG GGTTATTCG 19

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTACCCAATG CCTCAACCG 19

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 22 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GAGAACTGAT AGAAATTGGA TG 22

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 18 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGGACATGAG GTTCTCCG 18

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGGCTGTGTG AATCCTCAG 19

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGGTTCACCA CTGTCTCGTC 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 18 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCCAGGATGC TCTCCTCG 18

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAAGTCCTGG TAGCAAAGTC 20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATGGCAAGGT CAAAGAGCG 19

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 22 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CAACAATGTA TTCAGNAAGT CC 22

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TTGATACAAC ACTTTGTATC G 21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGAATACTAT CAGAAGGCAA G 21

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ACAGAGCAAG TTACTCAGAT G 21

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GTACACAATG CAGGCATTAG 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AATGTGGATG TTAATGTGCA C 21

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTGACCTCGT CTTCCTAC 19

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CAGCAAGATG AGGAGATGC 19

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGAAATGGTG GAAGATGATT C 21

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 16BASE PAIRS

(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTTCTCAACA CCAAGC 16

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GAAATTGATG AGGAAGGGAA C 21

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 22 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CTTCTGATTG ACAACTATGT GC 22

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 22 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CACAGAAGAT GGAAATATCC TG 22

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTGTTGGTAG CACTTAAGAC 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TTTCCCATAT TCTTCACTTG 20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GTAACATGAG CCACATGGC 19

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CCACTGTCTC GTCCAGCCG 19

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 26 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGGGATCCAT GTCGTTCGTG GCAGGG 26

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 26 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCTCTAGATT AACACCTCTC AAAGAC 26

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GCATCTAGAC GTTTCCTTGG C 21

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CATCCAAGCT TCTGTTCCCG 20

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GGGGTGCAGC AGCACATCG 19

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GGAGGCAGAA TGTGTGAGCG 20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TCCCAAAGAA GGACTTGCT 19

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 22 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

AGTATAAGTC TTAAGTGCTA CC 22

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TTTATGGTTT CTCACCTGCC 20

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GTTATCTGCC CACCTCAGC 19

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 59 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GGATCCTAAT ACGACTCACT ATAGGGAGAC CACCATGGCA TCTAGACGTT TCCCTTGGC 59

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CATCCAAGCT TCTGTTCCCG 20

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 56 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GGATCCTAAT ACGACTCACT ATAGGGAGAC CACCATGGGG GTGCAGCAGC ACATCG 56

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GGAGGCAGAA TGTGTGAGCG 20

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 28 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CGGGATCCAT GAAACAATTG CCTGCGGC 28

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 26 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GCTCTAGACC AGACTCATGC TGTTTT 26

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 26 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CGGGATCCAT GGAGCGAGCT GAGAGC 26

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 23 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GCTCTAGAGT GAAGACTCTG TCT 23

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS

(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

AAGCTGCTCT GTTAAAAGCG 20

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 18 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GCACCAGCAT CCAAGGAG 18

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CAACCATGAG ACACATCGC 19

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

AGGTTAGTGA AGACTCTGTC 20

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 53 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GGATCCTAAT ACGACTCACT ATAGGGAGAC CACCATGGAA CAATTGCCTG CGG 53

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 18 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

CCTGCTCCAC TCATCTGC 18

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 60 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GGATCCTAAT ACGACTCACT ATAGGGAGAC CACCATGGAA GATATCTTAA AGTTAATCCG 60

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GGCTTCTTCT ACTCTATATG G 21

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 58 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGATCCTAAT ACGACTCACT ATAGGGAGAC CACCATGGCA GGTCTTGAAA ACTCTTCG 58

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

AAAACAAGTC AGTGAATCCT C 21

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

AAGCACATCT GTTTCTGCTG 20

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

ACGAGTAGAT TCCTTTAGGC 20

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

CAGAACTGAC ATGAGAGCC
19

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 52 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GGATCCTAAT ACGACTCACT ATAGGGAGAC CACCATGGAG CGAGCTGAGA GC 52

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

AGGTTAGTGA AGACTCTGTC 20

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 17 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CTGAGGTCTC AGCAGGC 17

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 57 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

GGATCCTAAT ACGACTCACT ATAGGGAGAC CACCATGGTG TCCATTTCCA GACTGCG 57

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

AGGTTAGTGA AGACTCTGTC 20

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

TTATTTGGCA GAAAAGCAGA G 21

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

TTAAAAGACT AACCTCTTGC C 21

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CTGCTGTTAT GAACAATATG G 21

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

CAGAAGCAGT TGCAAAGCC 19

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

AAACCGTACT CTTCACACAC 20

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 20 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GAGGAAAAGC TTTTGTTGGC 20

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 18 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CAGTGGCTGC TGACTGAC 18

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 19 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

TCCAGAACCA AGAAGGAGC 19

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 16 BASE PAIRS

(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

TGAGGTCTCA GCAGGC 16

WHAT IS CLAIMED IS:

1. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having the deduced amino acid sequence of SEQ ID No. 2 or a fragment, analog or derivative of said polypeptide;

(b) a polynucleotide encoding a polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75649;

(c) a polynucleotide encoding a polypeptide having the deduced amino acid sequence of SEQ ID No. 4 or a fragment, analog or derivative of said polypeptide;

(d) a polynucleotide encoding a polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75651;

(e) a polynucleotide encoding a polypeptide having the deduced amino acid sequence of SEQ ID No. 6 or a fragment, analog or derivative of said polypeptide; and

(f) a polynucleotide encoding a polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75650.

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.

5. The polynucleotide sequence of claim 1 for use in analyzing a sample for mutation of a polynucleotide sequence encoding a human mismatch repair protein comprising:

a polynucleotide sequence of at least 15 and no more than 30 consecutive bases of the polynucleotide sequence of ATTC Deposit No. 75649.

6. The polynucleotide sequence of claim 1 for use in analyzing a sample for mutation of a polynucleotide sequence encoding a human mismatch repair protein comprising:

a polynucleotide sequence of at least 15 and no more than 30 consecutive bases of the the polynucleotide sequence of ATTC Deposit No. 75651.

7. The polynucleotide sequence of claim 1 for use in analyzing a sample for mutation of a polynucleotide sequence encoding a human mismatch repair protein comprising:

a polynucleotide sequence of at least 15 and no more than 30 consecutive bases of the the polynucleotide sequence of ATTC Deposit No. 75650.

8. The polynucleotide of Claim 2 wherein said polynucleotide encodes a polypeptide having the deduced amino acid sequence of SEQ ID No. 2.

9. The polynucleotide of Claim 2 wherein said polynucleotide encodes a polypeptide having the deduced amino acid sequence of SEQ ID No. 4.

10. The polynucleotide of Claim 2 wherein said polynucleotide encodes a polypeptide having the deduced amino acid sequence of SEQ ID No. 6.

11. The polynucleotide of Claim 2 wherein said polynucleotide encodes a polypeptide encoded by the cDNA of ATCC Deposit No. 75649.

12. The polynucleotide of Claim 2 wherein said polynucleotide encodes a polypeptide encoded by the cDNA of ATCC Deposit No. 75651.

13. The polynucleotide of Claim 2 wherein said polynucleotide encodes a polypeptide encoded by the cDNA of ATCC Deposit No. 75650.

14. The polynucleotide of Claim 1 having the coding sequence of SEQ ID No. 1.

15. The polynucleotide of Claim 1 having the coding sequence of SEQ ID No. 3.

16. The polynucleotide of Claim 1 having the coding sequence of SEQ ID No. 5).

17. A vector containing the DNA of Claim 2.

18. A host cell genetically engineered with the vector of Claim 17.

19. A process for producing a polypeptide comprising: expressing from the host cell of Claim 18 the polypeptide encoded by said DNA.

20. A process for producing cells capable of expressing a polypeptide comprising genetically engineering cells with the vector of Claim 17.

21. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having hMLH1 activity.

22. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having hMLH2 activity.

23. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having hMLH3 activity.

24. A polypeptide selected from the group consisting of:

(a) a polypeptide having the deduced amino acid sequence of SEQ ID No. 2 and fragments, analogs and derivatives thereof;

(b) a polypeptide encoded by the cDNA of ATCC Deposit No. 75649 and fragments, analogs and derivatives of said polypeptide;

(c) a polypeptide having the deduced amino acid sequence of SEQ ID No. 4 and fragments, analogs and derivatives thereof;

(d) a polypeptide encoded by the cDNA of ATCC Deposit No. 75651 and fragments, analogs and derivatives of said polypeptide;

(e) a polypeptide having the deduced amino acid sequence of SEQ ID No. 6 and fragments, analogs and derivatives thereof; and

(f) a polypeptide encoded by the cDNA of ATCC Deposit No. 75650 and fragments, analogs and derivatives of said polypeptide.

25. The polypeptide of Claim 15 wherein the polypeptide is hMLH1 having the deduced amino acid sequence of SEQ ID No. 2.

26. The polypeptide of Claim 14 wherein the polypeptide is hMLH2 having the deduced amino acid sequence of SEQ ID No. 4.

27. The polypeptide of Claim 14 wherein the polypeptide is hMLH3 having the deduced amino acid sequence of SEQ ID No. 6.

28. A process for diagnosing a susceptibility to cancer comprising:

determining from a sample derived from a human patient a mutation in a human mismatch repair gene, said human mismatch repair gene comprising the polynucleotide sequence of claim 8.

29. A process for diagnosing a susceptibility to cancer comprising:

determining from a sample derived from a human patient a mutation in a human mismatch repair gene, said human mismatch repair gene comprising the DNA of claim 9.

30. A process for diagnosing a susceptibility to cancer comprising:

determining from a sample derived from a human patient a mutation in a human mismatch repair gene, said human mismatch repair gene comprising the DNA of claim 10.

31. A process for diagnosing a susceptibility to cancer comprising:

determining from a sample derived from a human patient a mutation in a human DNA mismatch repair gene which encodes the human homolog of a bacterial mutL DNA mismatch repair gene.

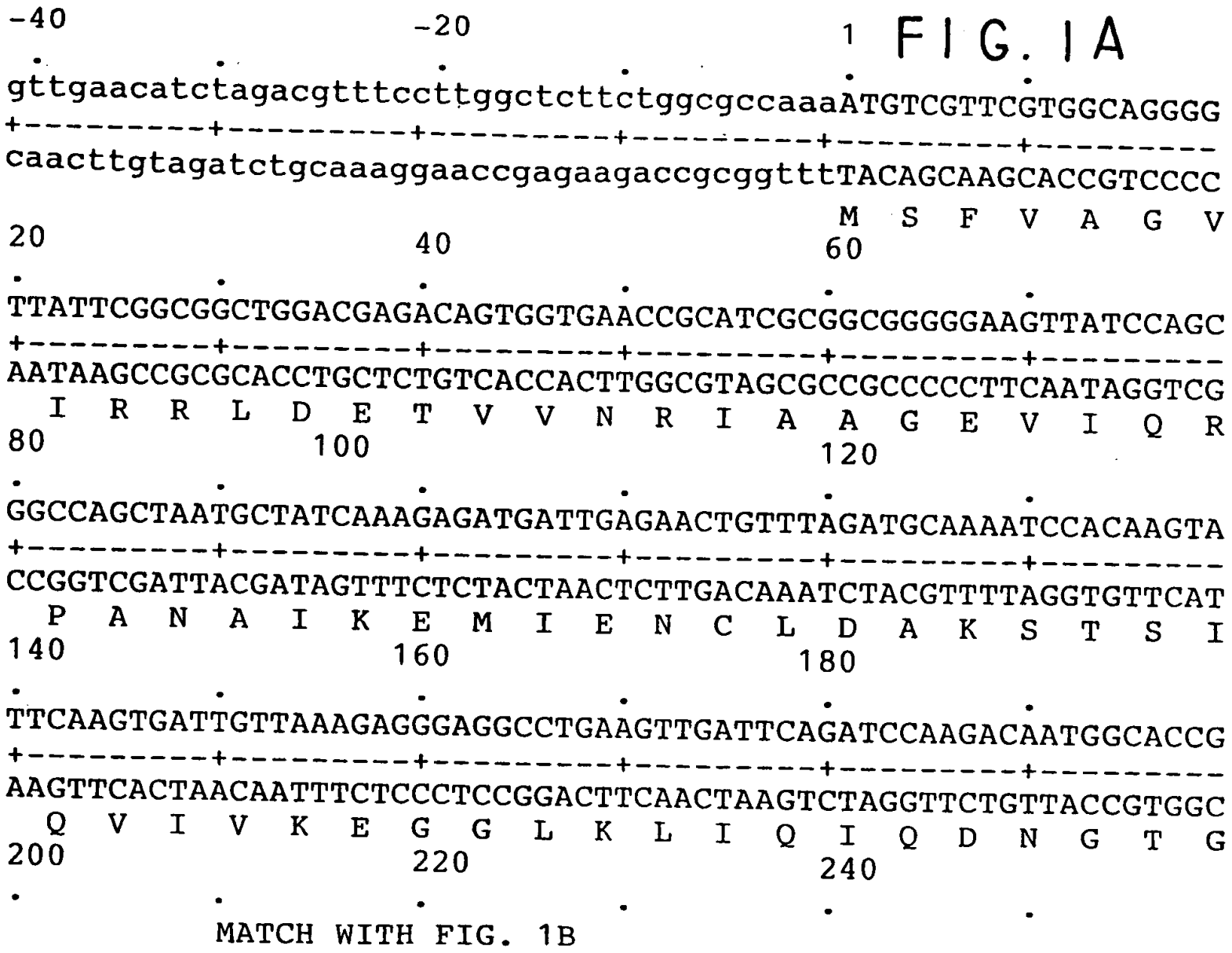
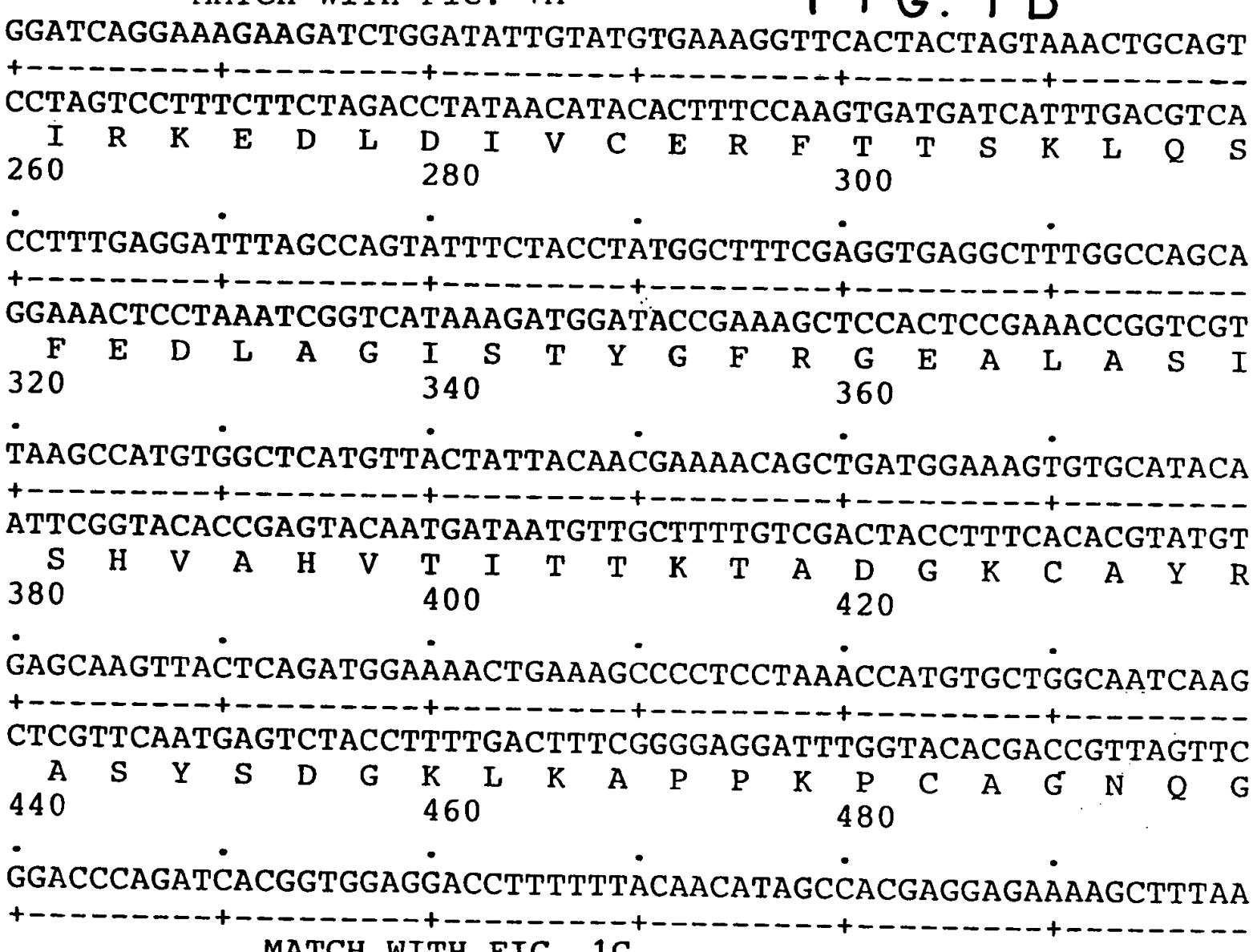


FIG. 1A

MATCH WITH FIG. 1A

FIG. 1B



MATCH WITH FIG. 1C

MATCH WITH FIG. 1B

FIG. 1C

```
CCTGGGTCTAGTGCCACCTCCTGGAAAAAATGTTGTATCGGTGCTCCTCTTTTCGAAATT
  T  Q  I  T  V  E  D  L  F  Y  N  I  A  T  R  R  K  A  L  K
500                  520                  540

.         .         .         .         .         .
AAAATCCAAGTGAAGAATATGGGAAAATTTTGGAAGTTGTTGCCAGGTATTCAGTACACA
+---------+---------+---------+---------+---------+---------
TTTTAGGTTCACTTCTTATACCCTTTTAAAACCTTCAACAACCGTTCATAAGTCATGTGT
  N  P  S  E  E  Y  G  K  I  L  E  V  V  G  R  Y  S  V  H  N
560                  580                  600

.         .         .         .         .         .
ATGCAGGCATTAGTTTCTCAGTTAAAAAACAAGGAGAGACAGTAGCTGATGTTAGGACAC
+---------+---------+---------+---------+---------+---------+---------
TACGTCCGTAATCAAAGAGTCAATTTTTTGTTCCTCTCTGTCATCGACTACAATCCTGTG
  A  G  I  S  F  S  V  K  K  Q  G  E  T  V  A  D  V  R  T  L
620                  640                  660

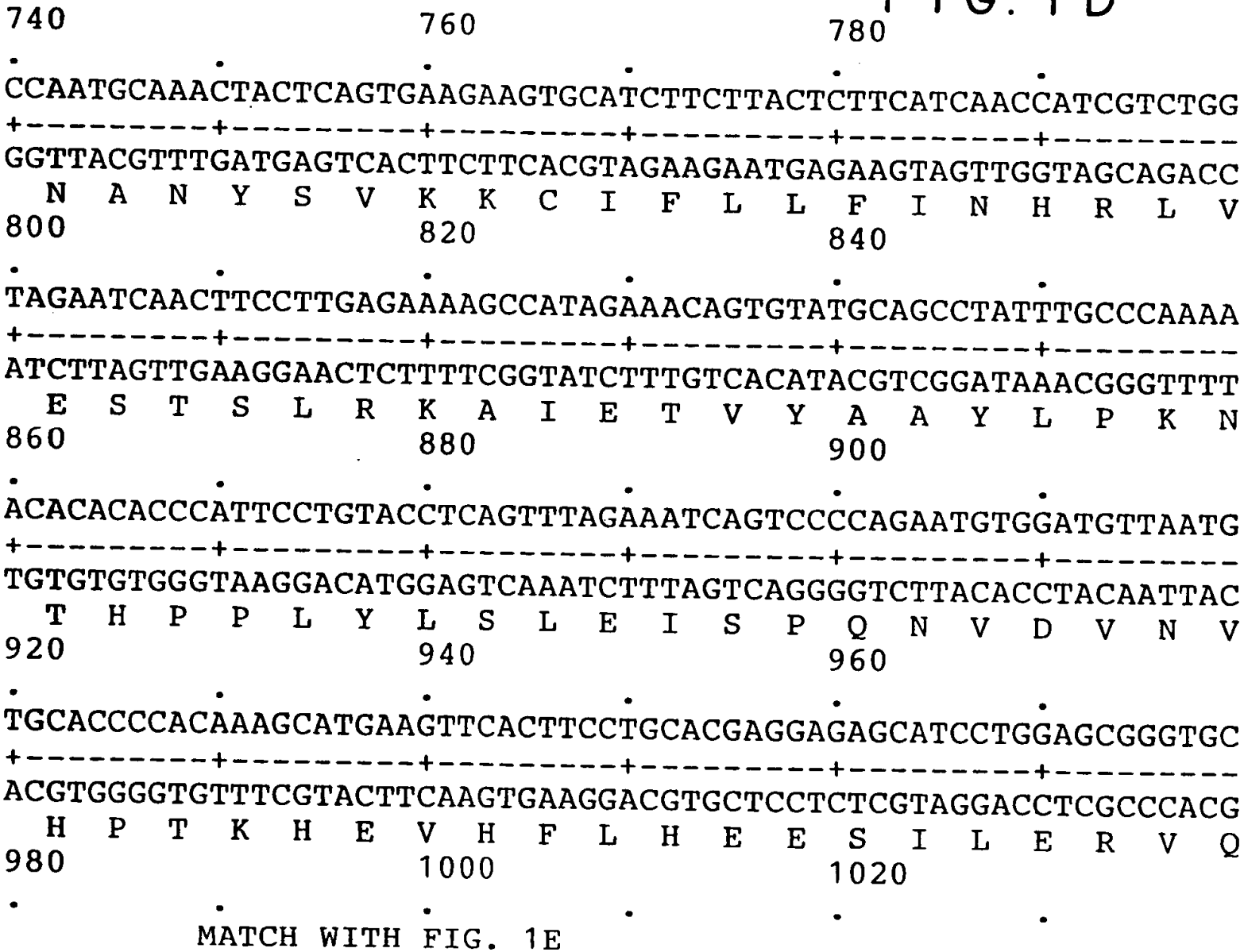
.         .         .         .         .         .
TACCCAATGCCTCAACCGTGGACAATATTCGCTCCGTCTTTGGAAATGCTGTTAGTCGAG
+---------+---------+---------+---------+---------+---------+---------
ATGGGTTACGGAGTTGGCACCTGTTATAAGCGAGGCAGAAACCCTTACGACAATCACCTC
  P  N  A  S  T  V  D  N  I  R  S  V  F  G  N  A  V  S  R  E
680                  700                  720

.         .         .         .         .         .
AACTGATAGAAATTGGATGTGAGGATAAAACCCTAGCCTTCAAAATGAATGGTTACATAT
+---------+---------+---------+---------+---------+---------+---------
TTGACTATCTTTAACCTACACTCCTATTTTGGGATCGGAAGTTTTACTTACCAATGTATA
  L  I  E  I  G  C  E  D  K  T  L  A  F  K  M  N  G  Y  I  S
```

MATCH WITH FIG. 1D

MATCH WITH FIG. 1C

FIG. 1D



MATCH WITH FIG. 1E

MATCH WITH FIG. 1D

FIG. 1E

```
AGCAGCACATCGAGAGCAAGCTCCTGGGCTCCAATTCCTCCAGGATGTACTTCACCCAGA
+---------+---------+---------+---------+---------+---------+---------
TCGTCGTGTAGCTCTCGTTCGAGGACCCGAGGTTAAGGAGGTCCTACATGAAGTGGGTCT
  Q   H   I   E   S   K   L   L   G   S   N   S   S   R   M   Y   F   T   Q   T
1040                    1060                    1080
.         .         .         .         .         .
CTTTGCTACCAGGACTTGCTGGCCCCTCTGGGGAGATGGTTAAATCCACAACAAGTCTCA
+---------+---------+---------+---------+---------+---------+---------
GAAACGATGGTCCTGAACGACGGGGGAGACCCCTCTACCAATTTAGGTGTTGTTCAGACT
  L   L   H   G   L   A   A   P   S   G   E   M   V   K   S   T   T   S   L   T
1100                    1120                    1140
.         .         .         .         .         .
CCTCGTCTTCTACTTCTGGAAGTAGTGATAAGGTCTATGCCCACCAGATGGTTCGTACAG
+---------+---------+---------+---------+---------+---------+---------
GGAGCAGAAGATGAAGACCTTCATCACTATTCCAGATACGGGTGGTCTACCAAGCATGTC
  S   S   S   T   S   G   S   S   D   K   V   Y   A   H   Q   M   V   R   T   D
1160                    1180                    1200
.         .         .         .         .         .
ATTCCCGGGAACAGAAGCTTGATGCATTTCTGCAGCCTCTGAGCAAACCCCTGTCCAGTC
+---------+---------+---------+---------+---------+---------+---------
TAAGGGCCCTTGTCTTCGAACTACGTAAAGACGTCGGAGACTCGTTTGGGGACAGGTCAG
  S   R   E   Q   K   I   D   A   F   L   Q   P   L   S   K   P   L   S   S   Q
1280                    1240                    1260
.         .         .         .         .         .
AGCCCCAGGCCATTGTCACAGAGGATAAGACAGATATTTCTAGTGGCAGGGCTAGGCAGC
```

MATCH WITH FIG. 1F

MATCH WITH FIG. 1E

FIG. 1F

```
TCGGGGTCCGGTAACAGTGTCTCCTATTCTGTCTATAAAGATCACCGTCCCGATCCGTCG
  P  Q  A  I  V  T  E  D  K  T  D  I  S  S  G  R  A  R  Q  Q
1280                1300                1320
.         .         .         .         .         .
AAGATGAGGAGATGCTTGAACTCCCAGCCCCTGCTGAAGTGGCTGCCAAAAATCAGAGCT
TTCTACTCCTCTACGAACTTGAGGGTCGGGGACGACTTCACCGACGGTTTTTAGTCTCGA
  D  E  E  M  L  E  L  P  A  P  A  E  V  A  A  K  N  Q  S  L
1340                1360                1380
.         .         .         .         .         .
TGGAGGGGGATACAACAAAGGGGACTTCAGAAATGTCAGAGAAGAGAGGACCTACTTCCA
ACCTCCCCCTATGTTGTTTCCCCTGAAGTCTTTACAGTCTCTTCTCTCCTGGATGAAGGT
  E  G  D  T  T  K  G  T  S  E  M  S  E  K  R  G  P  T  S  S
1400                1420                1440
.         .         .         .         .         .
GCAACCCCAGAAAGAGACATCGGGAAGATTCTGATCTCCAAATCCTCGAAGATGATTCCC
CGTTGGGGTCTTTCTCTGTAGCCCTTCTAAGACTACACCTTTACCACCTTCTACTAAGGG
  N  P  R  K  R  H  R  E  D  S  D  V  E  M  V  E  D  D  S  R
1460                1480                1500
.         .         .         .         .         .
GAAAGGAAATGACTGCAGCTTGTACCCCCCGGAGAAGGATCATTAACCTCACTAGTGTTT
CTTTCCTTTACTGACGTCGAACATGGGGGGCCTCTTCCTAGTAATTGGAGTGATCACAAA
```

MATCH WITH FIG. 1G

MATCH WITH FIG. 1F

FIG. 1G

```
  K  E  M  T  A  A  C  T  P  R  R  R  I  I  N  L  T  S  V  L
1520                 1540                 1560
.     .     .     .     .     .     .     .     .     .     .
TGAGTCTCCAGGAAGAAATTAATGAGCAGGGACATGAGGTTCTCCGGGAGATGTTGCATA
+---------+---------+---------+---------+---------+---------+---------
ACTCAGAGGTCCTTCTTTAATTACTCGTCCCTGTACTCCAAGAGGCCCTCTACAACGTAT
  S  L  Q  E  E  I  N  E  Q  G  H  E  V  L  R  E  M  L  H  N
1580                 1600                 1620
.     .     .     .     .     .     .     .     .     .     .
ACCACTCCTTCGTGGGCTGTGTGAATCCTCAGTGGGCCTTGGCACAGCATCAAACCAAGT
+---------+---------+---------+---------+---------+---------+---------
TGGTGAGGAAGCACCCGACACACTTAGGAGTCACCCGGAACCGTGTCGTAGTTTGCTTCA
  H  S  F  V  G  C  V  N  P  Q  W  A  L  A  Q  H  Q  T  K  L
1640                 1660                 1680
.     .     .     .     .     .     .     .     .     .     .
TATACCTTCTCAACACCACCAAGCTTAGTGAAGAACTGTTCTACCAGATACTCATTTATG
+---------+---------+---------+---------+---------+---------+---------
ATATGGAAGAGTTGTGGTGGTTCGAATCACTTCTTGACAAGATGGTCTATGAGTAAATAC
  Y  L, L  N  T  T  K  L  S  E  E  L  F  Y  Q  I  L  I  Y  D
1700                 1720                 1740
.     .     .     .     .     .     .     .     .     .     .
ATTTTGCCAATTTTGGTGTTCTCAGGTTATCGGAGCCAGCACCGCTCTTTGACCTTGCCA
+---------+---------+---------+---------+---------+---------+---------
TAAAACGGTTAAAACCACAAGAGTCCAATAGCCTCGGTCGTGGCGAGAAACTGGAACGGT
  F  A  N  F  G  V  L  R  L  S  E  P  A  P  L  F  D  L  A  M
1760                 1780                 1800
```

MATCH WITH FIG. 1H

FIG. 1H

MATCH WITH FIG. 1G

```
TGCTTCCCTTAGATAGTCCAGAGAGTGGCTGGACAGAGGAAGATGGTCCCAAAGAAGGAC
+---------+---------+---------+---------+---------+---------+---------
ACGAACGGAATCTATCAGGTCTCTCACCGACCTGTCTCCTTCTACCAGGGTTTCTTCCTG
  L  A  L  D  S  P  E  S  G  W  T  E  E  D  G  P  K  E  G  L
1820                 1840                 1860

TTGCTGAATACATTGTTGAGTTTCTGAAGAAGAAGGCTGAGATGCTTGCAGACTATTTCT
+---------+---------+---------+---------+---------+---------+---------
AACGACTTATGTAACAACTCAAAGACTTCTTCTTCCGACTCTACGAACGTCTGATAAAGA
  A  E  Y  I  V  E  F  L  K  K  K  A  E  M  L  A  D  Y  F  S
1880                 1900                 1920

CTTTGGAAATTGATGAGGAAGGGAACCTGATTGGATTACCCCTTCTGATTGACAACTATG
+---------+---------+---------+---------+---------+---------+---------
GAAACCTTTAACTACTCCTTCCCTTGGACTAACCTAATGGGGAAGACTAACTGTTGATAC
  L  E  I  D  E  E  G  N  L  I  G  L  P  L  L  T  D  N  Y  V
1940                 1960                 1980

TGCCCCCTTTGGAGGGACTCCCTATCTTCATTCTTCCACTAGCCACTGAGGTGAATTGGG
+---------+---------+---------+---------+---------+---------+---------
ACGGGGGAAACCTCCCTGACGGATAGAAGTAAGAAGCTGATCGGTGACTCCACTTAACCC
  P  P  L  E  G  L  P  I  F  I  L  R  L  A  T  E  V  N  W  D
2000                 2020                 2040

ACGAAGAAAAGGAATGTTTTGAAAGCCTCAGTAAAGAATGCGCTATGTTCTATTCCATCC
```

MATCH WITH FIG. 1I

MATCH WITH FIG. 1H

FIG. II

```
+---------+---------+---------+---------+---------+---------+---------
TGCTTCTTTTCCTTACAAAACTCTCGGAGTCATTTCTTACGCGATACAAGATAAGGTAGG
  E   E   K   E   C   F   E   S   L   S   K   E   C   A   M   F   Y   S   I   R
2060                    2080                    2100
.         .         .         .         .         .
GGAAGCAGTACATATCTGAGGAGTCGACCCTCTCAGGCCAGCAGAGTGAAGTGCCTGGCT
+---------+---------+---------+---------+---------+---------+---------
CCTTCGTCATGTATAGACTCCTCAGCTGGGAGAGTCCGGTCGTCTCACTTCACGGACCGA
  K   Q   Y   I   S   E   E   S   T   L   S   G   Q   Q   S   E   V   P   G   S
2120                    2140                    2160
.         .         .         .         .         .
CCATTCCAAACTCCTGGAAGTGGACTGTGGAACACATTGTCTATAAAGCCTTGCGCTCAC
+---------+---------+---------+---------+---------+---------+---------
GGTAAGGTTTGAGGACCTTCACCTGACACCTTGTGTAACAGATATTTCGGAACGCGAGTG
  I   P   N   S   W   K   W   T   V   E   H   I   V   Y   K   A   L   R   S   H
2180                    2200                    2220
.         .         .         .         .         .
ACATTCTGCCTCCTAAACATTTCACAGAAGATGGAAATATCCTGCAGCTTGCTAACCTGC
+---------+---------+---------+---------+---------+---------+---------
TGTAAGACGGAGGATTTGTAAAGTGTCTTCTACCTTTATAGGACGTCGAACGATTGGACG
  I   L   P   P   K   H   S   T   E   D   G   N   I   L   Q   L   A   N   L   P
2240                    2260                    2280
.         .         .         .         .         .
CTGATCTATACAAAGTCTTTGAGAGGTGTTAAATATGGTTATTTATGCACTGTGGGATGT
+---------+---------+---------+---------+---------+---------+---------
GACTAGATATGTTTCAGAAACTCTCCACAATTTATACCAATAAATACGTGACACCCTACA
```

MATCH WITH IG. 1J

SUBSTITUTE SHEET (RULE 26)

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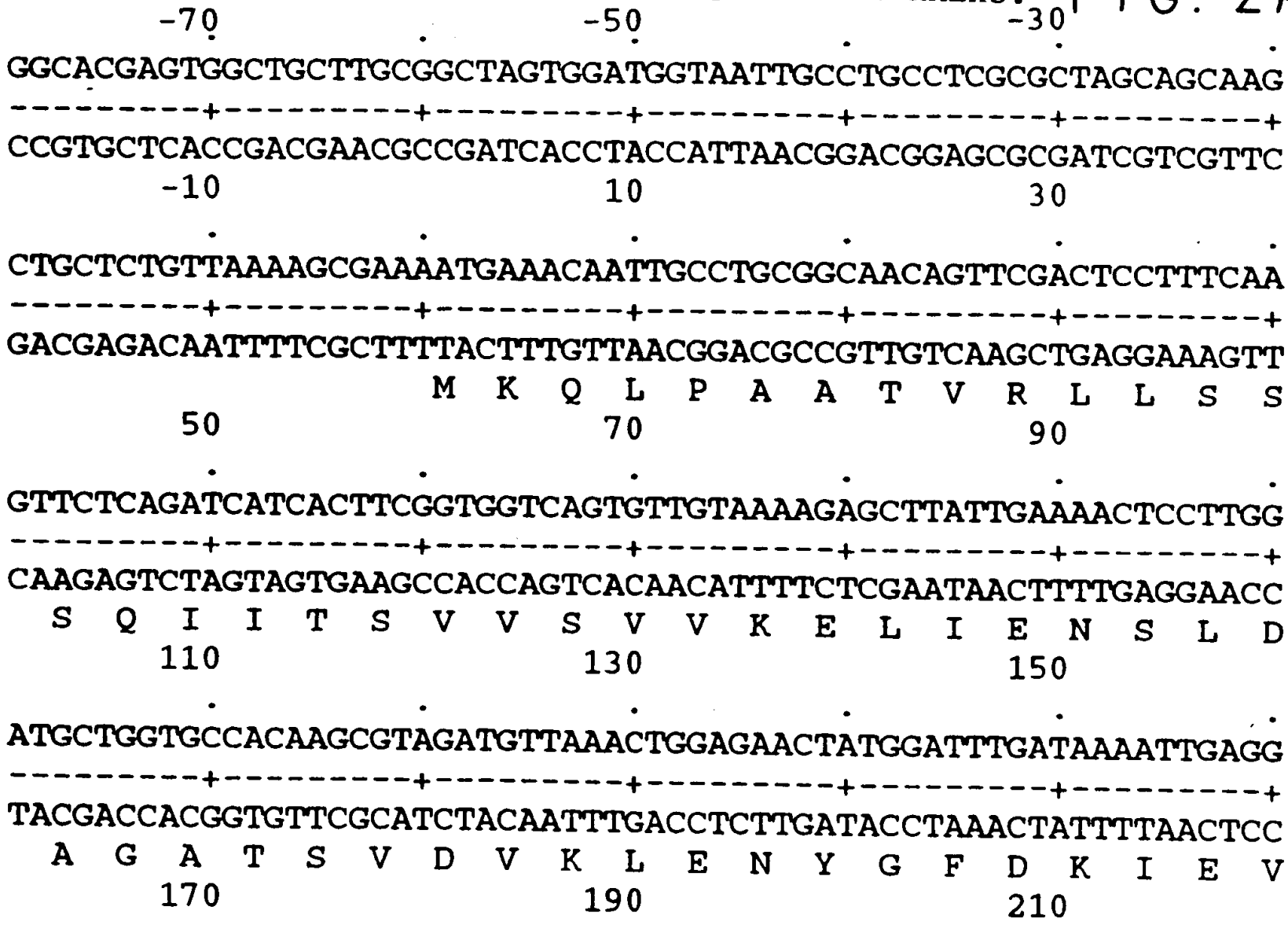
FIG. 1J

MATCH WITH FIG. 1I

```
  D  L  Y  K  V  F  E  R  C  *
2300                2320                2340
.         .         .         .         .         .
GTTCTTCTTTCTCTGTATTCCGATACAAAGTGTTGTATCAAAGTGTGATATACAAAGTGT
+---------+---------+---------+---------+---------+---------+---------
CAAGAAGAAAGAGACATAAGGCTATGTTTCACAACATAGTTTCACACTATATGTTTCACA
2360                2380                2400
.         .         .         .         .         .
ACCAACATAAGTGTTGGTAGCACTTAAGACTTATACTTGCCTTCTGACAGTATTCCTTTA
+---------+---------+---------+---------+---------+---------+---------
TGGTTGTATTCACAACCATCGTGAATTCTGAATATGAACGGAAGACTATCATAAGGAAAT
2420                2440                2460
.         .         .         .         .         .
TACACATGTGATTGATTATAAATAAATAGATGTGTCTTAACATAAAAAAAAAAAAAAAA
+---------+---------+---------+---------+---------+---------+---------
ATGTGTCACCTAACTAATATTTATTTATCTACACAGAATTGTATTTTTTTTTTTTTTTT
2480
.
AAAAA
+----
TTTTT
```

Polynucleotide and deduced amino acid sequence of hMLH3: FIG. 2A



MATCH WITH FIG. 2B

MATCH WITH FIG. 2A

FIG. 2B

```
TGCGAGATAACGGGGAGGGTATCAAGGCTGTTGATGCACCTGTAATGGCAATGAAGTACT
---------+---------+---------+---------+---------+---------+
ACGCTCTATTGCCCCTCCCATAGTTCCGACAACTACGTGGACATTACCGTTACTTCATGA
  R  D  N  G  E  G  I  K  A  V  D  A  P  V  M  A  M  K  Y  Y
        230                     250                     270

ACACCTCAAAAATAAATAGTCATGAAGATCTTGAAAATTTGACAACTTACGGTTTTCGTG
---------+---------+---------+---------+---------+---------+
TGTGGAGTTTTTATTTATCAGTACTTCTAGAACTTTTAAACTGTTGAATGCCAAAAGCAC
  T  S  K  I  N  S  H  E  D  L  E  N  L  T  T  Y  G  F  R  G
        290                     310                     330

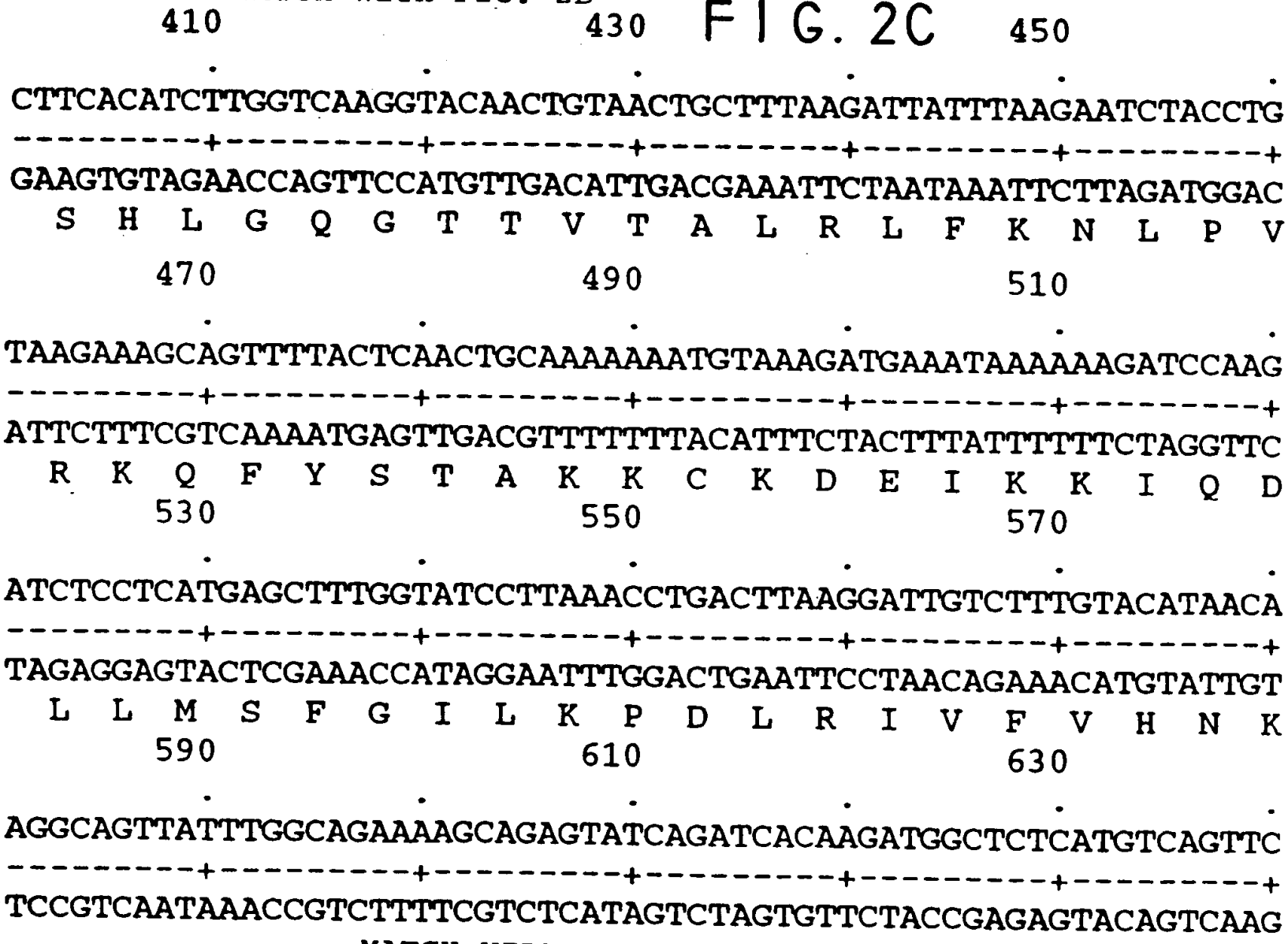
GAGAAGCCTTGGGGTCAATTTGTTGTATAGCTGAGGTTTTAATTACAACAAGAACGGCTG
---------+---------+---------+---------+---------+---------+
CTCTTCGGAACCCCAGTTAAACAACATATCGACTCCAAAATTAATGTTGTTCTTGCCGAC
  E  A  L  G  S  I  C  C  I  A  E  V  L  I  T  T  R  T  A  A
        350                     370                     390

CTGATAATTTTAGCACCCAGTATGTTTTAGATGGCAGTGGCCACATACTTTCTCAGAAAC
---------+---------+---------+---------+---------+---------+
GACTATTAAAATCGTGGGTCATACAAAATCTACCGTCACCGGTGTATGAAAGAGTCTTTG
  D  N  F  S  T  Q  Y  V  L  D  G  S  G  H  I  L  S  Q  K  P
```

MATCH WITH FIG. 2C

MATCH WITH FIG. 2B

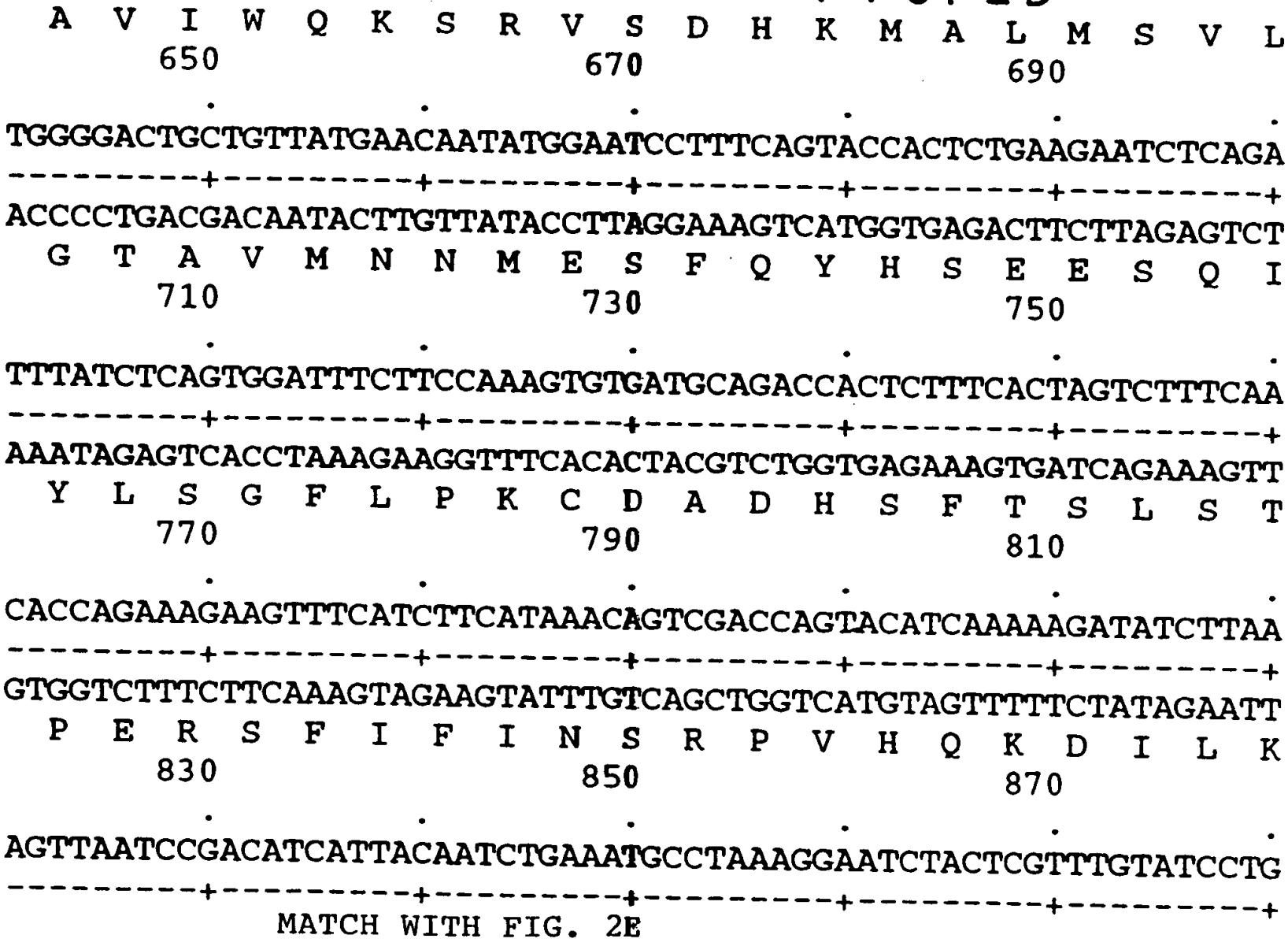
FIG. 2C



MATCH WITH FIG. 2D

MATCH WITH FIG. 2C

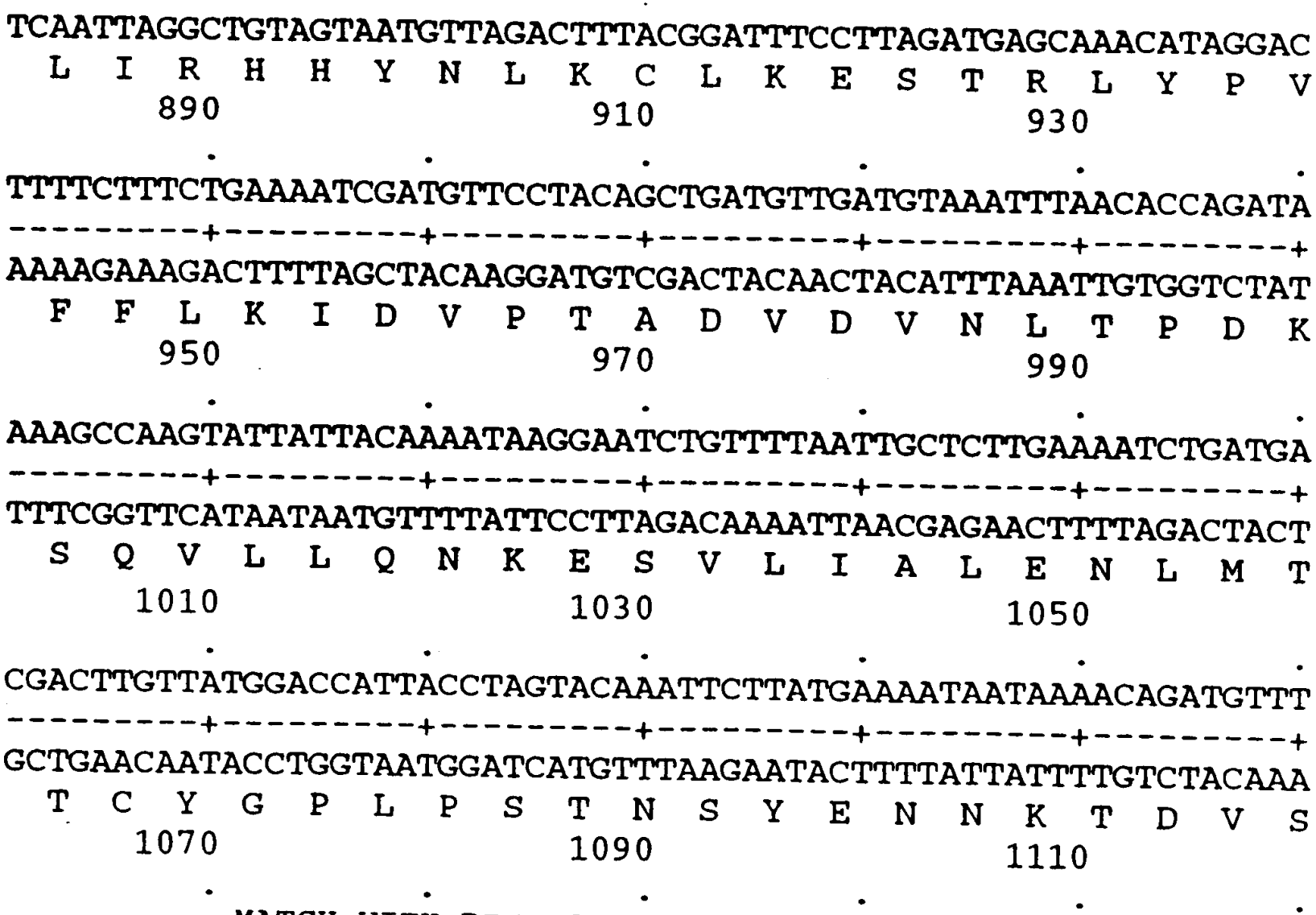
FIG. 2D



MATCH WITH FIG. 2E

MATCH WITH FIG. 2D

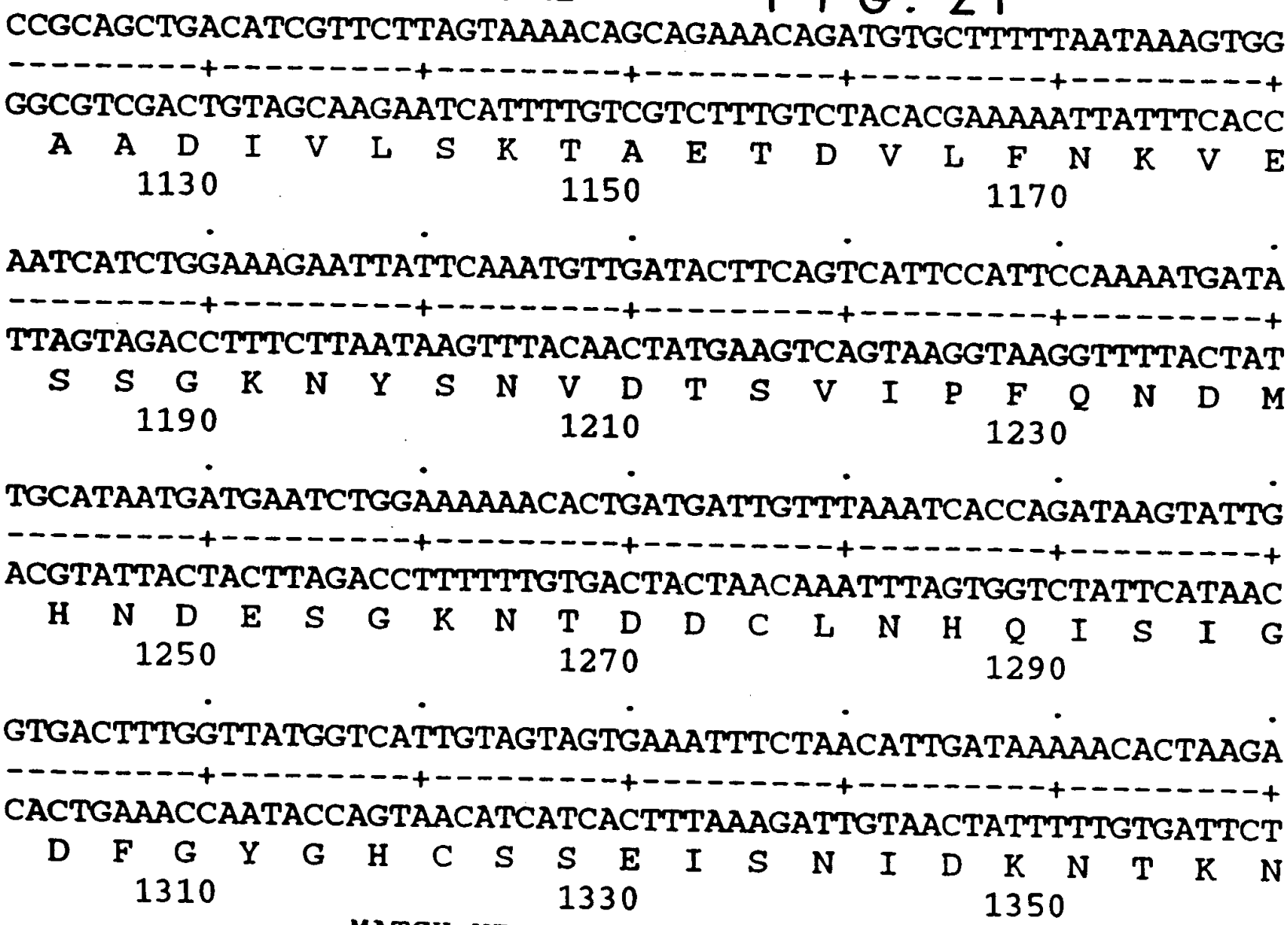
FIG. 2E



MATCH WITH FIG. 2F

MATCH WITH FIG. 2E

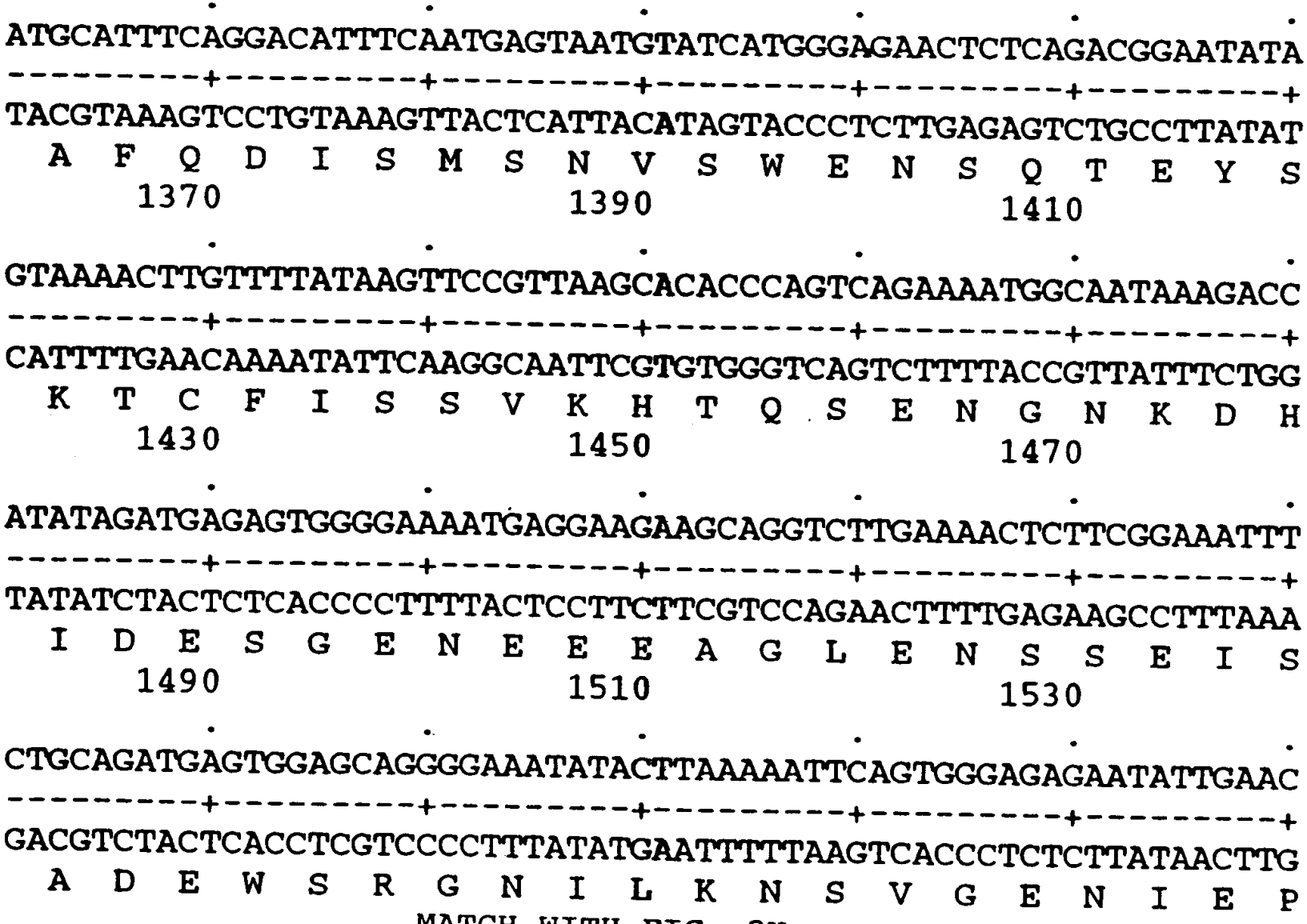
FIG. 2F



MATCH WITH FIG. 2G

MATCH WITH FIG. 2F

FIG. 2G



MATCH WITH FIG. 2H

MATCH WITH FIG. 2G

FIG. 2H

```
      1550                1570                1590
       .         .         .         .         .
CTGTGAAAATTTTAGTGCCTGAAAAAAGTTTACCATGTAAAGTAAGTAATAATAATTATC
---------+---------+---------+---------+---------+---------+
GACACTTTTAAAATCACGGACTTTTTTCAAATGGTACATTTCATTCATTATTATTAATAG
  V   K   I   L   V   P   E   K   S   L   P   C   K   V   S   N   N   N   Y   P
      1610                1630                1650
       .         .         .         .         .
CAATCCCTGAACAAATGAATCTTAATGAAGATTCATGTAACAAAAAATCAAATGTAATAG
---------+---------+---------+---------+---------+---------+
GTTAGGGACTTGTTTACTTAGAATTACTTCTAAGTACATTGTTTTTTAGTTTACATTATC
  I   P   E   Q   M   N   L   N   E   D   S   C   N   K   K   S   N   V   I   D
      1670                1690                1710
       .         .         .         .         .
ATAATAAATCTGGAAAAGTTACAGCTTATGATTTACTTAGCAATCGAGTAATCAAGAAAC
---------+---------+---------+---------+---------+---------+
TATTATTTAGACCTTTTCAATGTCGAATACTAAATGAATCGTTAGCTCATTAGTTCTTTG
  N   K   S   G   K   V   T   A   Y   D   L   L   S   N   R   V   I   K   K   P
      1730                1750                1770
       .         .         .         .         .
CCATGTCAGCAAGTGCTCTTTTTGTTCAAGATCATCGTCCTCAGTTTCTCATAGAAAATC
---------+---------+---------+---------+---------+---------+
GGTACAGTCGTTCACGAGAAAAACAAGTTCTAGTAGCAGGAGTCAAAGAGTATCTTTTAG
```

MATCH WITH FIG. 2I

MATCH WITH FIG. 2H

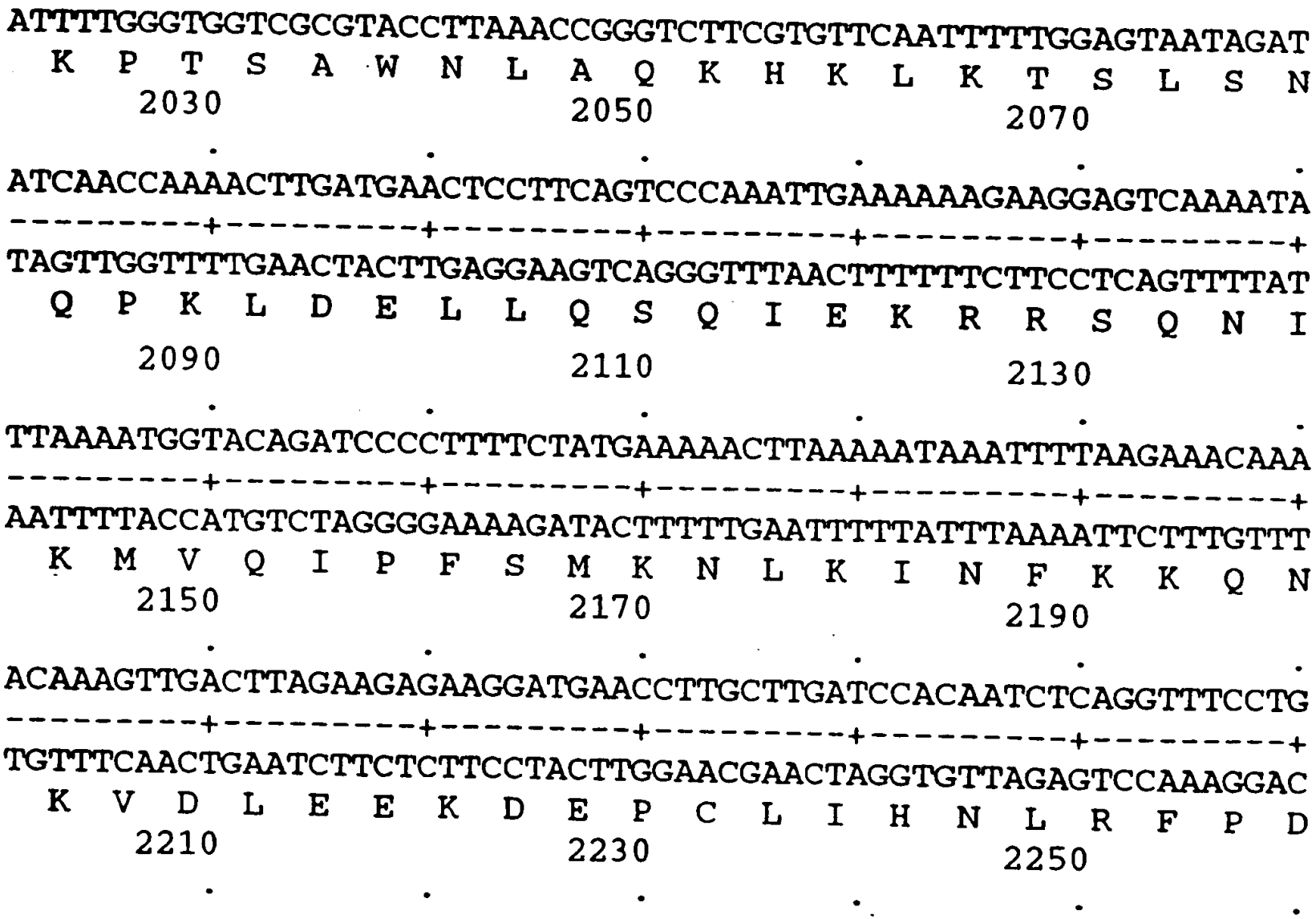
FIG. 2I

```
M  S  A  S  A  L  F  V  Q  D  H  R  P  Q  F  L  I  E  N  P
      1790                 1810                 1830
       .     .     .     .     .     .     .     .     .
CTAAGACTAGTTTAGAGGATGCAACACTACAAATTGAAGAACTGTGGAAGACATTGAGTG
---------+---------+---------+---------+---------+---------+---------+
GATTCTGATCAAATCTCCTACGTTGTGATGTTTAACTTCTTGACACCTTCTGTAACTCAC
  K  T  S  L  E  D  A  T  L  Q  I  E  E  L  W  K  T  L  S  E
      1850                 1870                 1890
       .     .     .     .     .     .     .     .     .
AAGAGGAAAAACTGAAATATGAAGAGAAGGCTACTAAAGACTTGGAACGATACAATAGTC
---------+---------+---------+---------+---------+---------+---------+
TTCTCCTTTTTGACTTTATACTTCTCTTCCGATGATTTCTGAACCTTGCTATGTTATCAG
  E  E  K  L  K  Y  E  E  K  A  T  K  D  L  E  R  Y  N  S  Q
      1910                 1930                 1950
       .     .     .     .     .     .     .     .     .
AAATGAAGAGAGCCATTGAACAGGAGTCACAAATGTCACTAAAAGATGGCAGAAAAAAGA
---------+---------+---------+---------+---------+---------+---------+
TTTACTTCTCTCGGTAACTTGTCCTCAGTGTTTACAGTGATTTTCTACCGTCTTTTTTCT
  M  K  R  A  I  E  Q  E  S  Q  M  S  L  K  D  G  R  K  K  I
      1970                 1990                 2010
       .     .     .     .     .     .     .     .     .
TAAAACCCACCAGCGCATGGAATTTGGCCCAGAAGCACAAGTTAAAAACCTCATTATCTA
---------+---------+---------+---------+---------+---------+---------+
```

MATCH WITH FIG. 2J

MATCH WITH FIG. 2I

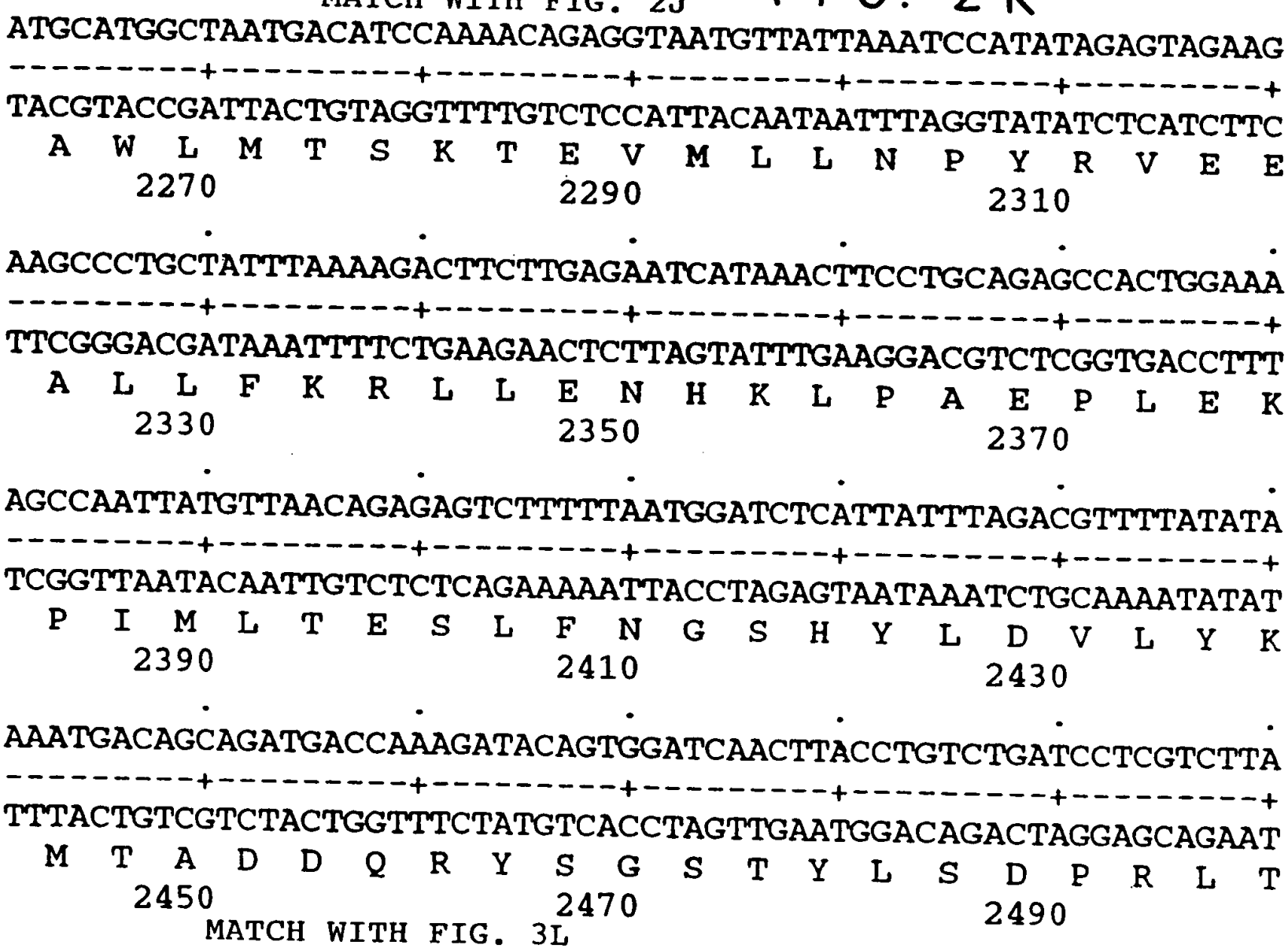
FIG. 2J



MATCH WITH FIG. 2K

MATCH WITH FIG. 2J

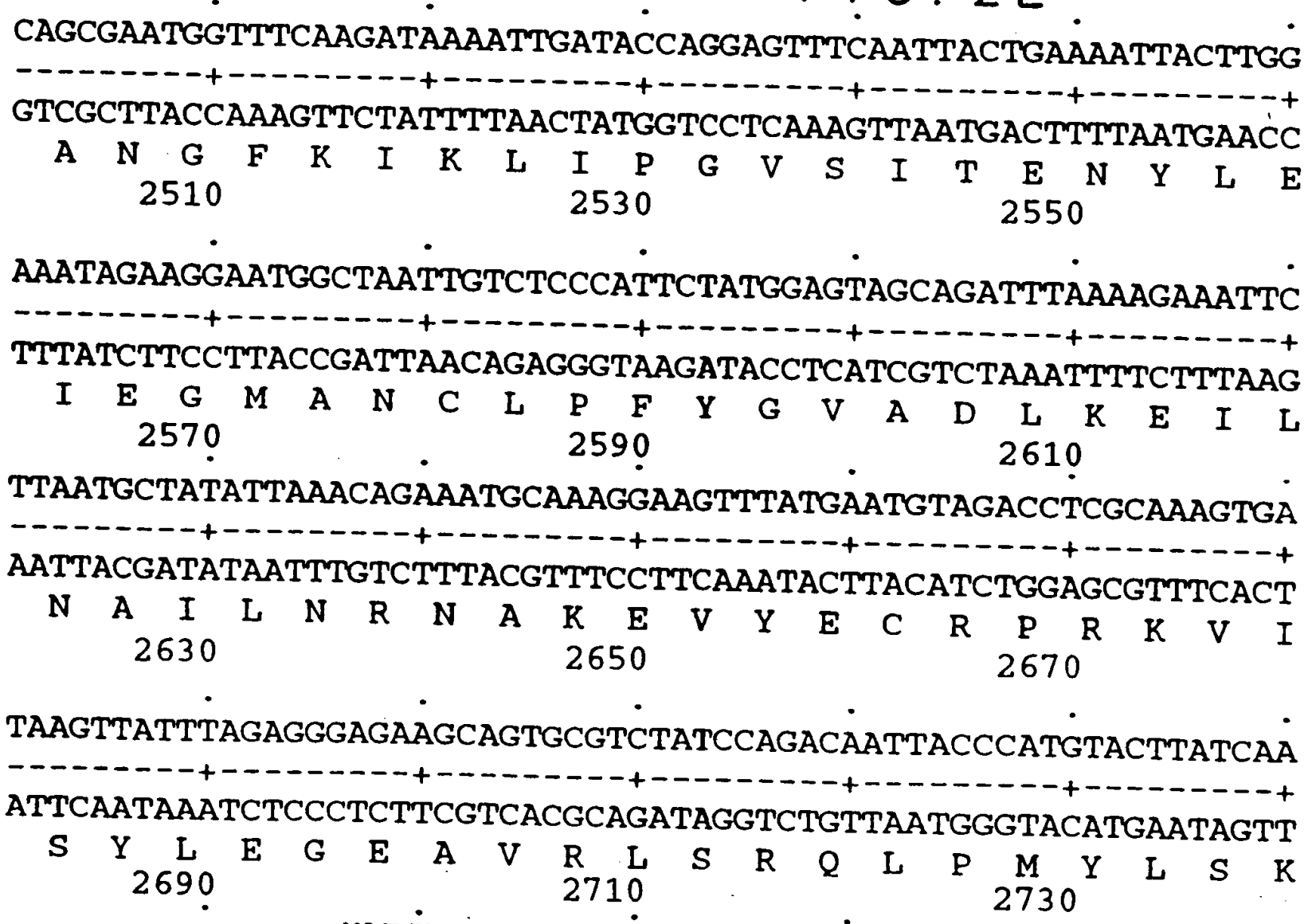
FIG. 2K



MATCH WITH FIG. 3L

MATCH WITH FIG. 2K

FIG. 2L



MATCH WITH FIG. 2M

MATCH WITH FIG. 2L

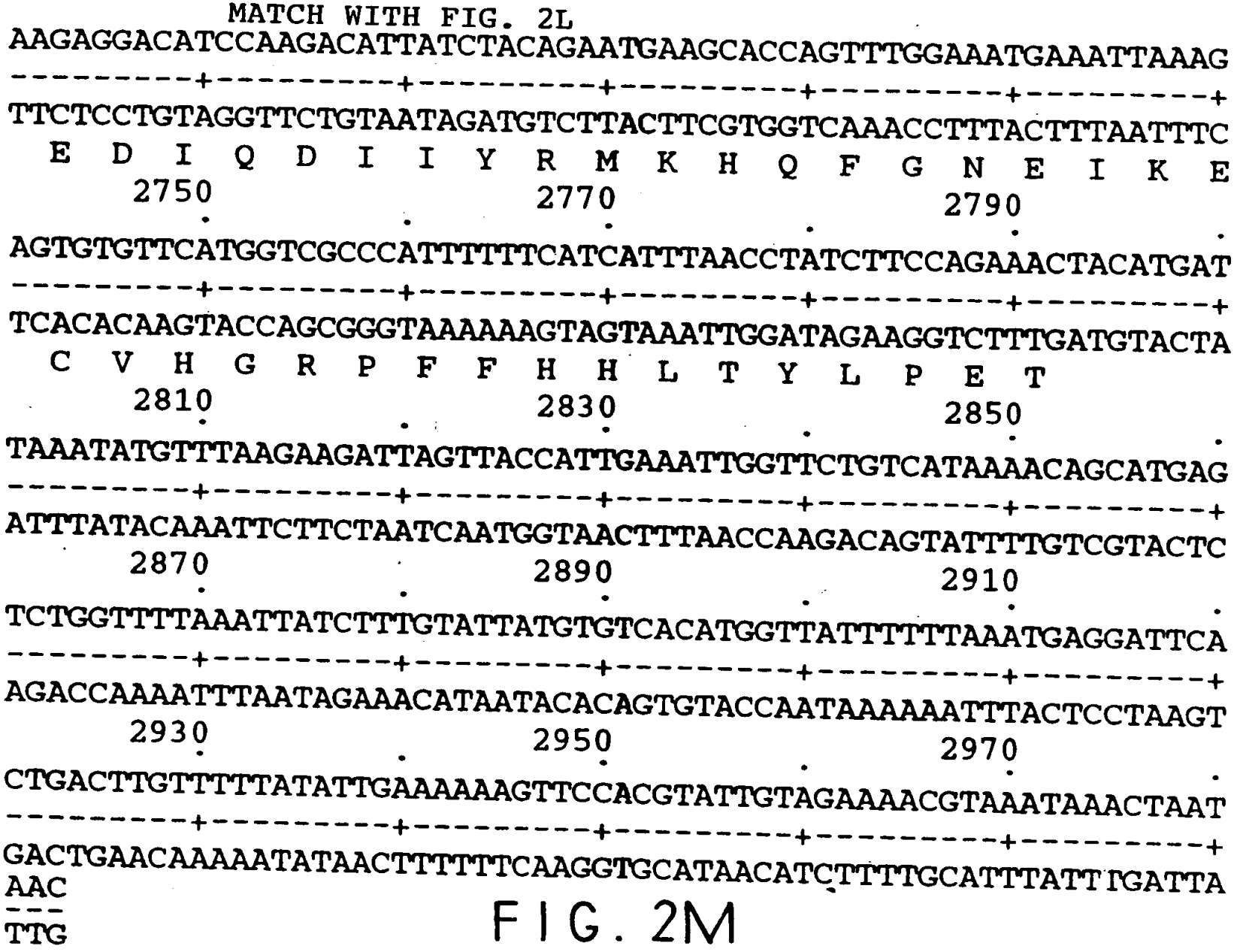


FIG. 2M

Polynucleotide and deduced amino acid sequence of hMLH2:

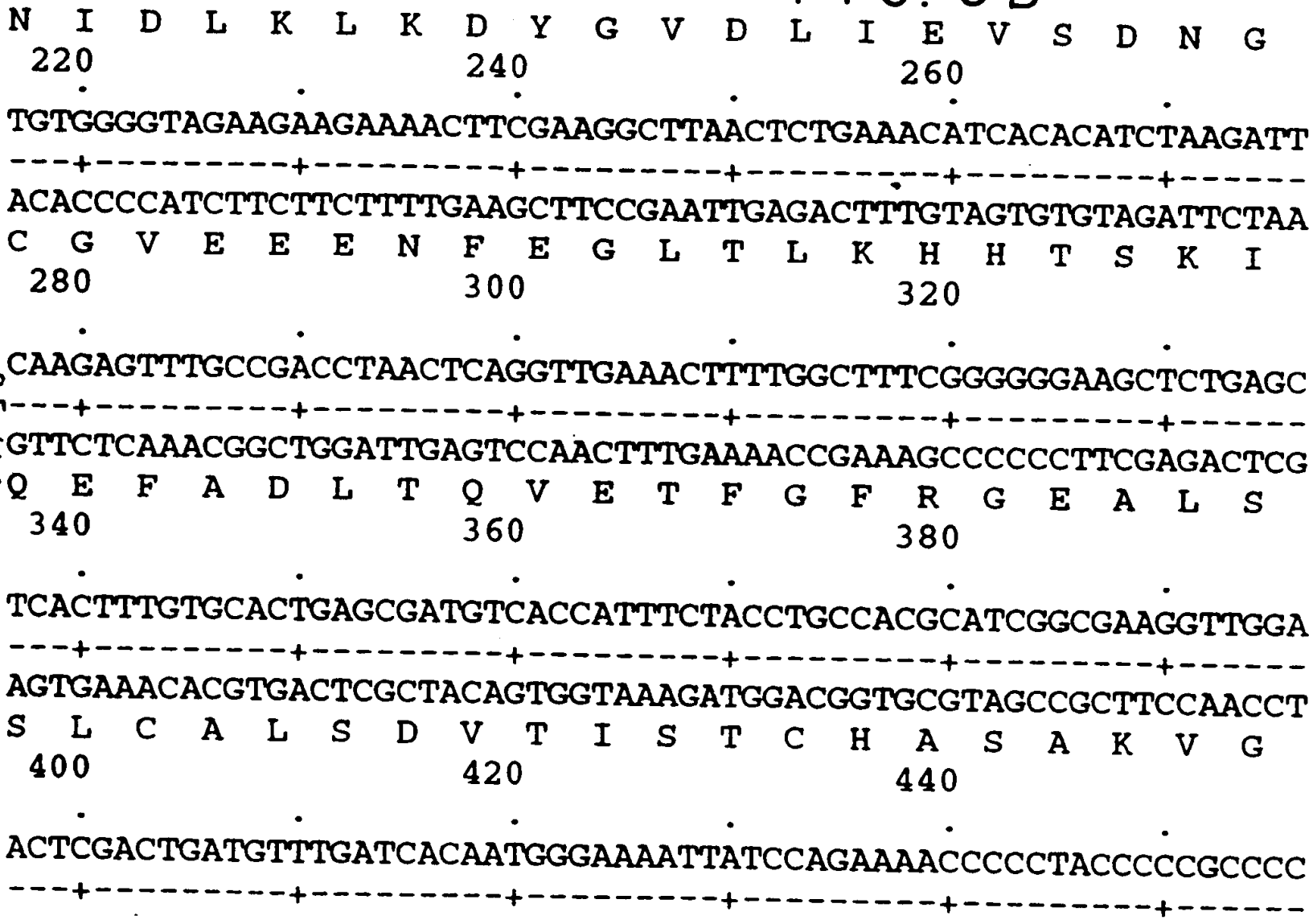
FIG. 3A

```
-20                    0                   20
 .         .         .         .         .         .
CGAGGCGGATCGGGTGTTGCATCCATGGAGCGAGCTGAGAGCTCGAGTACAGAACCTGCT
---+---------+---------+---------+---------+---------+---------+------
GCTCCGCCTAGCCCACAACGTAGGTACCTCGCTCGACTCTCGAGCTCATGTCTTGGACGA
                          M  E  R  A  E  S  S  S  T  E  P  A
  40                    60                  80
 .         .         .         .         .         .
AAGGCCATCAAACCTATTGATCGGAAGTCAGTCCATCAGATTTGCTCTGGGCAGGTGGTA
---+---------+---------+---------+---------+---------+---------+------
TTCCGGTAGTTTGGATAACTAGCCTTCAGTCAGGTAGTCTAAACGAGACCCGTCCACCAT
K  A  I  K  P  I  D  R  K  S  V  H  Q  I  C  S  G  Q  V  V
 100                   120                 140
 .         .         .         .         .         .
CTGAGTCTAAGCACTGCGGTAAAGGAGTTAGTAGAAAACAGTCTGGATGCTGGTGCCACT
---+---------+---------+---------+---------+---------+---------+------
GACTCAGATTCGTGACGCCATTTCCTCAATCATCTTTTGTCAGACCTACGACCACGGTGA
L  S  L  S  T  A  V  K  E  L  V  E  N  S  L  D  A  G  A  T
 160                   180                 200
 .         .         .         .         .         .
AATATTGATCTAAAGCTTAAGGACTATGGAGTGGATCTTATTGAAGTTTCAGACAATGGA
---+---------+---------+---------+---------+---------+---------+------
TTATAACTAGATTTCGAATTCCTGATACCTCACCTAGAATAACTTCAAAGTCTGTTACCT
```

MATCH WITH FIG. 3B

MATCH WITH FIG. 3A

FIG. 3B



MATCH WITH FIG. 3C

FIG. 3C

MATCH WITH FIG. 3B

```
TGAGCTGACTACAAACTAGTGTTACCCTTTTAATAGGTCTTTTGGGGATGGGGGCGGGG
 T  R  L  M  F  D  H  N  G  K  I  I  Q  K  T  P  Y  P  R  P
 460                480                 500

    .         .         .         .         .         .
AGAGGGACCACAGTCAGCGTGCAGCAGTTATTTTCCACACTACCTGTGCGCCATAAGGAA
---+---------+---------+---------+---------+---------+------
TCTCCCTGGTGTCAGTCGCACGTCGTCAATAAAAGGTGTGATGGACACGCGGTATTCCTT
 R  G  T  T  V  S  V  Q  Q  L  F  S  T  L  P  V  R  H  K  E
 520                540                 560

    .         .         .         .         .         .
TTTCAAAGGAATATTAAGAAGGAGTATGCCAAAATGGTCCAGGTCTTACATGCATACTGT
---+---------+---------+---------+---------+---------+------
AAAGTTTCCTTATAATTCTTCCTCATACGGTTTTACCAGGTCCAGAATGTACGTATGACA
 F  Q  R  N  I  K  K  E  Y  A  K  M  V  Q  V  L  H  A  Y  C
 580                600                 620

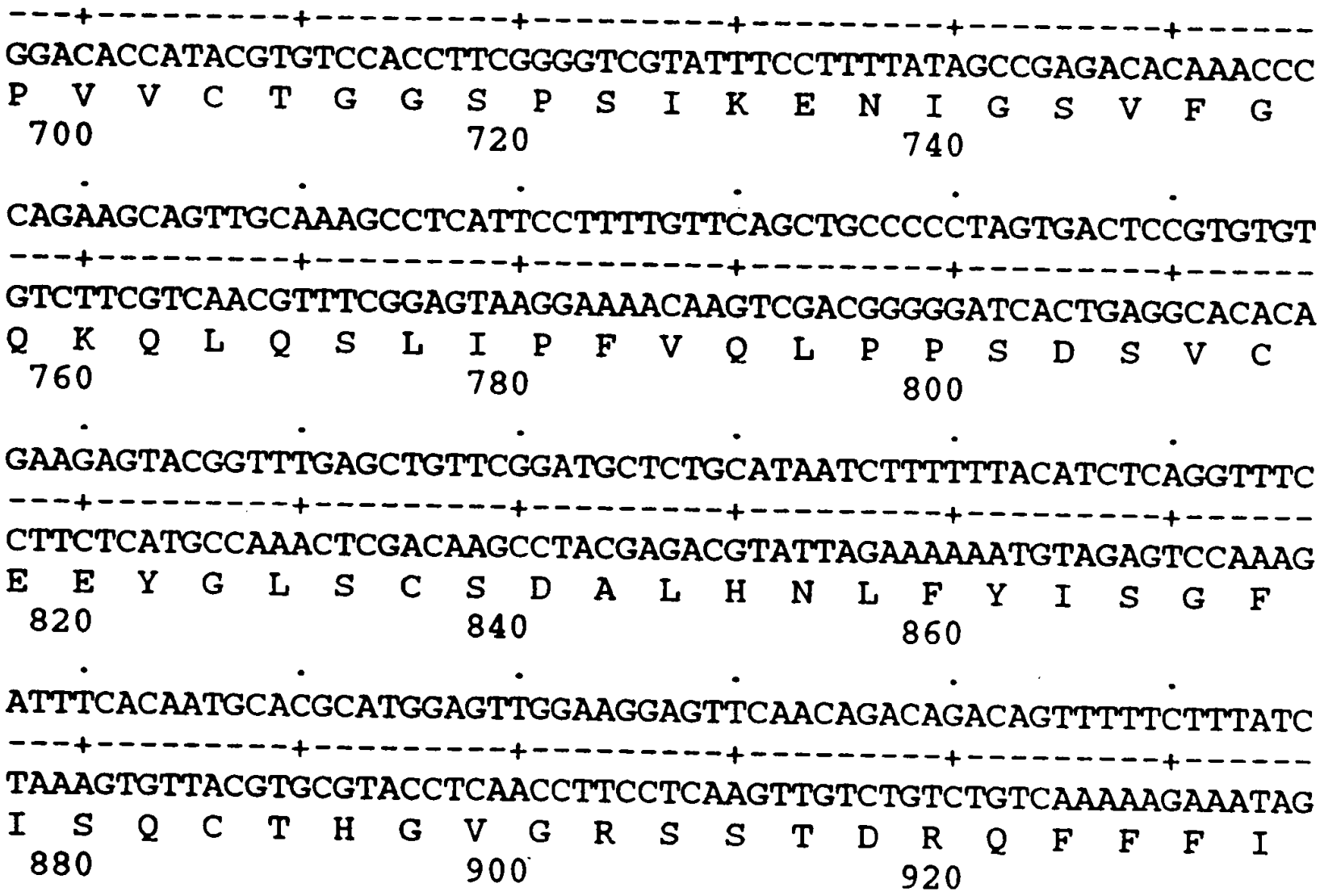
    .         .         .         .         .         .
ATCATTTCAGCAGGCATCCGTGTAAGTTGCACCAATCAGCTTGGACAAGGAAAACGACAG
---+---------+---------+---------+---------+---------+------
TAGTAAAGTCGTCCGTAGGCACATTCAACGTGGTTAGTCGAACCTGTTCCTTTTGCTGTC
 I  I  S  A  G  I  R  V  S  C  T  N  Q  L  G  Q  G  K  R  Q
 640                660                 680

    .         .         .         .         .         .
CCTGTGGTATGCACAGGTGGAAGCCCCAGCATAAAGGAAAATATCGGCTCTGTGTTTGGG
```

MATCH WITH FIG. 3D

MATCH WITH FIG. 3C

FIG. 3D



MATCH WITH FIG. 3E

FIG. 3E

MATCH WITH FIG. 3D

```
AACCGGCGGCCTTGTGACCCAGCAAAGGTCTGCAGACTCGTGAATGAGGTCTACCACATG
---+---------+---------+---------+---------+---------+------
TTGGCCGCCGGAACACTGGGTCGTTTCCAGACGTCTGAGCACTTACTCCAGATGGTGTAC
N  R  R  P  C  D  P  A  K  V  C  R  L  V  N  E  V  Y  H  M
 940                960                980

TATAATCGACACCAGTATCCATTTGTTGTTCTTAACATTTCTGTTGATTCAGAATGCGTT
---+---------+---------+---------+---------+---------+------
ATATTAGCTGTGGTCATAGGTAAACAACAAGAATTGTAAAGACAACTAAGTCTTACGCAA
Y  N  R  H  Q  Y  P  F  V  V  L  N  I  S  V  D  S  E  C  V
1000               1020               1040

GATATCAATGTTACTCCAGATAAAAGGCAAATTTTGCTACAAGAGGAAAAGCTTTTGTTG
---+---------+---------+---------+---------+---------+------
CTATAGTTACAATGAGGTCTATTTTCCGTTTAAAACGATGTTCTCCTTTTCGAAAACAAC
D  I  N  V  T  P  D  K  R  Q  I  L  L  Q  E  E  K  L  L  L
1060               1080               1100

GCAGTTTTAAAGACCTCTTTGATAGGAATGTTTGATAGTGATGTCAACAAGCTAAATGTC
---+---------+---------+---------+---------+---------+------
CGTCAAAATTTCTGGAGAAACTATCCTTACAAACTATCACTACAGTTGTTCGATTTACAG
A  V  L  K  T  S  L  I  G  M  F  D  S  D  V  N  K  L  N  V
```

MATCH WITH FIG. 3F

MATCH WITH FIG. 3E

FIG. 3F

```
1120                1140                1160
   .         .         .         .         .
AGTCAGCAGCCACTGCTGGATGTTGAAGGTAACTTAATAAAAATGCATGCAGCGGATTTG
---+---------+---------+---------+---------+---------+---------+------
TCAGTCGTCGGTGACGACCTACAACTTCCATTGAATTATTTTTACGTACGTCGCCTAAAC
S  Q  Q  P  L  L  D  V  E  G  N  L  I  K  M  H  A  A  D  L
1180                1200                1220
   .         .         .         .         .         .
GAAAAGCCCATGGTAGAAAAGCAGGATCAATCCCCTTCATTAAGGACTGGAGAAGAAAAA
---+---------+---------+---------+---------+---------+---------+------
CTTTTCGGGTACCATCTTTTCGTCCTAGTTAGGGGAAGTAATTCCTGACCTCTTCTTTTT
E  K  P  M  V  E  K  Q  D  Q  S  P  S  L  R  T  G  E  E  K
1240                1260                1280
   .         .         .         .         .         .
AAAGACGTGTCCATTTCCAGACTGCGAGAGGCCTTTTCTCTTCGTCACACAACAGAGAAC
---+---------+---------+---------+---------+---------+---------+------
TTTCTGCACAGGTAAAGGTCTGACGCTCTCCGGAAAAGAGAAGCAGTGTGTTGTCTCTTG
K  D  V  S  I  S  R  L  R  E  A  F  S  L  R  H  T  T  E  N
1300                1320                1340
   .         .         .         .         .         .
AAGCCTCACAGCCCAAAGACTCCAGAACCAAGAAGGAGCCCTCTAGGACAGAAAAGGGGT
---+---------+---------+---------+---------+---------+---------+------
TTCGGAGTGTCGGGTTTCTGAGGTCTTGGTTCTTCCTCGGGAGATCCTGTCTTTTCCCCA
```

MATCH WITH FIG. 3G

MATCH WITH FIG. 3F

FIG. 3G

```
K  P  H  S  P  K  T  P  E  P  R  R  S  P  L  G  Q  K  R  G
1360                 1380                 1400
.     .     .     .     .     .     .     .     .     .
ATGCTGTCTTCTAGCACTTCAGGTGCCATCTCTGACAAAGGCGTCCTGAGACCTCAGAAA
---+---------+---------+---------+---------+---------+---------+------
TACGACAGAAGATCGTGAAGTCCACGGTAGAGACTGTTTCCGCAGGACTCTGGAGTCTTT
M  L  S  S  S  T  S  G  A  I  S  D  K  G  V  L  R  P  Q  K
1420                 1440                 1460
.     .     .     .     .     .     .     .     .     .
GAGGCAGTGAGTTCCAGTCACGGACCCAGTGACCCTACGGACAGAGCGGAGGTGGAGAAG
---+---------+---------+---------+---------+---------+---------+------
CTCCGTCACTCAAGGTCAGTGCCTGGGTCACTGGGATGCCTGTCTCGCCTCCACCTCTTC
E  A  V  S  S  S  H  G  P  S  D  P  T  D  R  A  E  V  E  K
1480                 1500                 1520
.     .     .     .     .     .     .     .     .     .
GACTCGGGGCACGGCAGCACTTCCGTGGATTCTGAGGGGTTCAGCATCCCAGACACGGGC
---+---------+---------+---------+---------+---------+---------+------
CTGAGCCCCGTGCCGTCGTGAAGGCACCTAAGACTCCCCAAGTCGTAGGGTCTGTGCCCG
D  S  G  H  G  S  T  S  V  D  S  E  G  F  S  I  P  D  T  G
1540                 1560                 1580
.     .     .     .     .     .     .     .     .     .
AGTCACTGCAGCAGCGAGTATGCGGCCAGCTCCCCAGGGGACAGGGGCTCGCAGGAACAT
```

MATCH WITH FIG. 3H

MATCH WITH FIG. 3G

FIG. 3H

```
---+---------+---------+---------+---------+---------+---------+------
TCAGTGACGTCGTCGCTCATACGCCGGTCGAGGGGTCCCCTGTCCCCGAGCGTCCTTGTA
S  H  C  S  S  E  Y  A  A  S  S  P  G  D  R  G  S  Q  E  H
1600                  1620                  1640
   .         .         .         .         .         .

GTGGACTCTCAGGAGAAAGCGCCTGAAACTGACGACTCTTTTTCAGATGTGGACTGCCAT
---+---------+---------+---------+---------+---------+---------+------
CACCTGAGAGTCCTCTTTCGCGGACTTTGACTGCTGAGAAAAAGTCTACACCTGACGGTA
V  D  S  Q  E  K  A  P  E  T  D  D  S  F  S  D  V  D  C  H
1660                  1680                  1700
   .         .         .         .         .         .

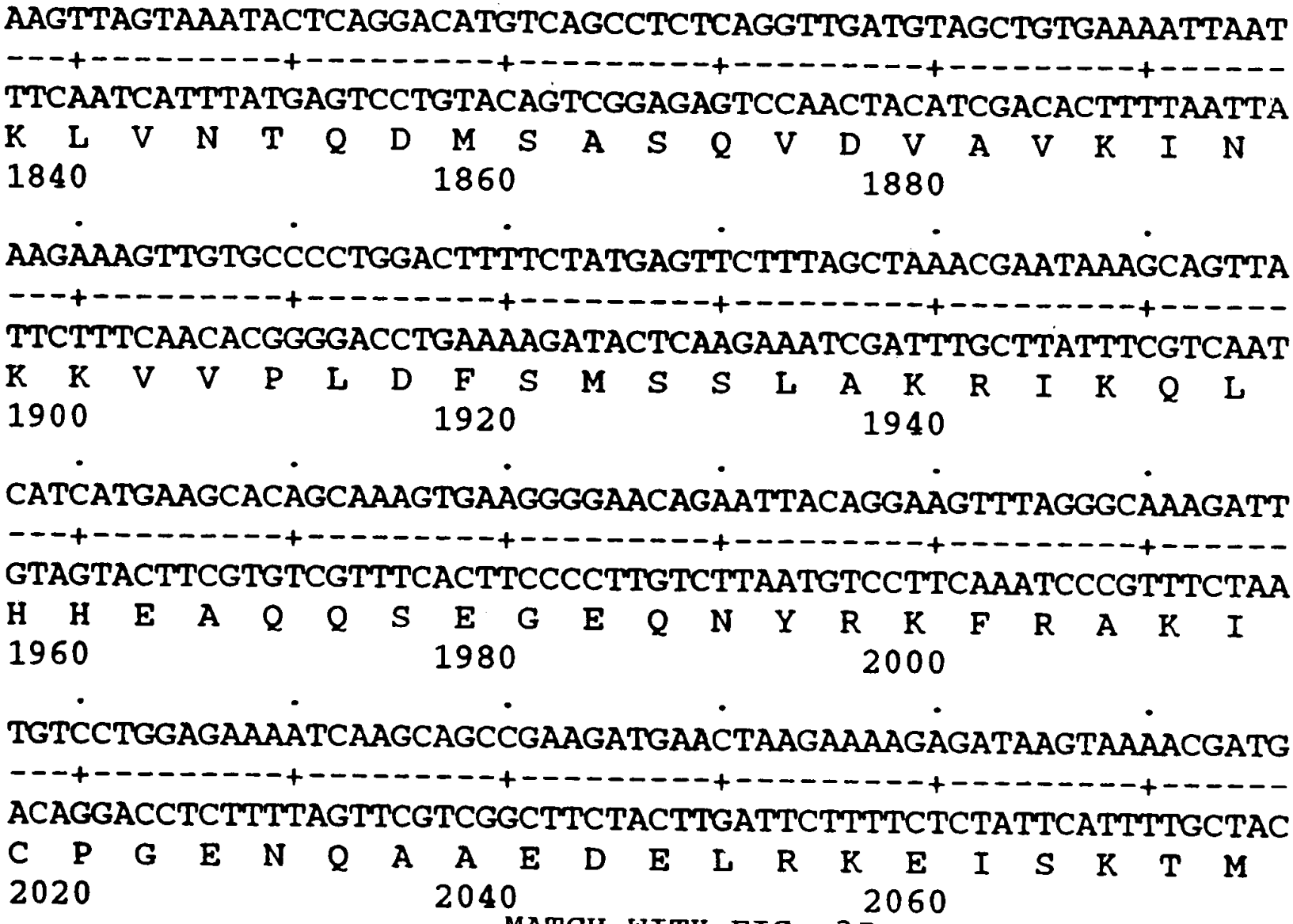
TCAAACCAGGAAGATACCGGATGTAAATTTCGAGTTTTGCCTCAGCCAACTAATCTCGCA
---+---------+---------+---------+---------+---------+---------+------
AGTTTGGTCCTTCTATGGCCTACATTTAAAGCTCAAAACGGAGTCGGTTGATTAGAGCGT
S  N  Q  E  D  T  G  C  K  F  R  V  L  P  Q  P  T  N  L  A
1720                  1740                  1760
   .         .         .         .         .         .

ACCCCAAACACAAAGCGTTTTAAAAAAGAAGAAATTCTTTCCAGTTCTGACATTTGTCAA
---+---------+---------+---------+---------+---------+---------+------
TGGGGTTTGTGTTTCGCAAAATTTTTTCTTCTTTAAGAAAGGTCAAGACTGTAAACAGTT
T  P  N  T  K  R  F  K  K  E  E  I  L  S  S  S  D  I  C  Q
1780                  1800                  1820
   .         .         .         .         .         .
```

MATCH WITH FIG. 3I

MATCH WITH FIG. 3H

FIG. 3I



MATCH WITH FIG. 3J

MATCH WITH FIG. 3I

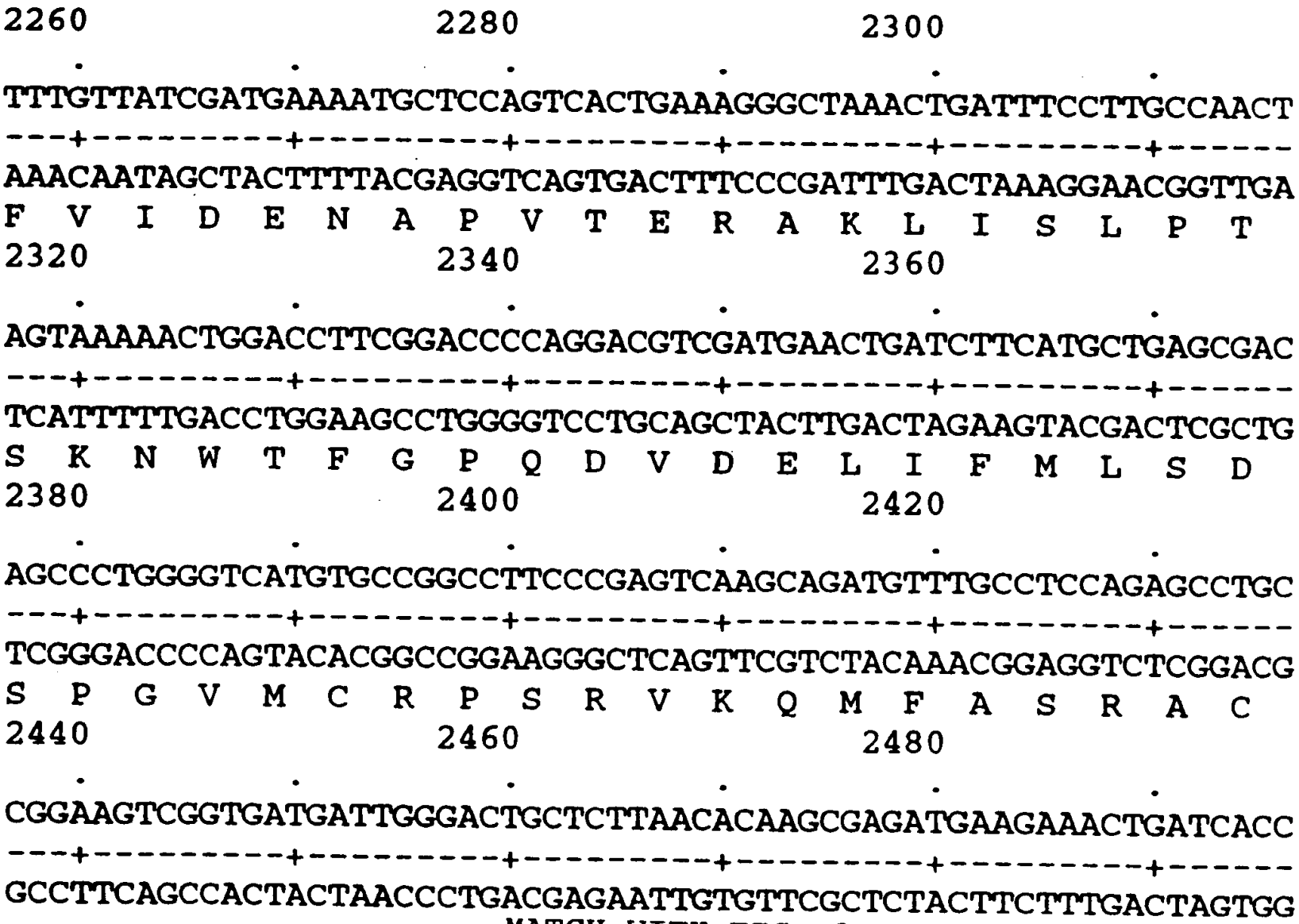
FIG. 3J

```
TTTGCAGAAATGGAAATCATTGGTCAGTTTAACCTGGGATTTATAATAACCAAACTGAAT
---+---------+---------+---------+---------+---------+---------+------
AAACGTCTTTACCTTTAGTAACCAGTCAAATTGGACCCTAAATATTATTGGTTTGACTTA
F  A  E  M  E  I  I  G  Q  F  N  L  G  F  I  I  T  K  L  N
2080                    2100                    2120
GAGGATATCTTCATAGTGGACCAGCATGCCACGGACGAGAAGTATAACTTCGAGATGCTG
---+---------+---------+---------+---------+---------+---------+------
CTCCTATAGAAGTATCACCTGGTCGTACGGTGCCTGCTCTTCATATTGAAGCTCTACGAC
E  D  I  F  I  V  D  Q  H  A  T  D  E  K  Y  N  F  E  M  L
2140                    2160                    2180
CAGCAGCACACCGTGCTCCAGGGGCAGAGGCTCATAGCACCTCAGACTCTCAACTTAACT
---+---------+---------+---------+---------+---------+---------+------
GTCGTCGTGTGGCACGAGGTCCCCGTCTCCGAGTATCGTGGAGTCTGAGAGTTGAATTGA
Q  Q  H  T  V  L  Q  G  Q  R  L  I  A  P  Q  T  L  N  L  T
2200                    2220                    2240
GCTGTTAATGAAGCTGTTCTGATAGAAAATCTGGAAATATTTAGAAAGAATGGCTTTGAT
---+---------+---------+---------+---------+---------+---------+------
CGACAATTACTTCGACAAGACTATCTTTTAGACCTTTATAAATCTTTCTTACCGAAACTA
A  V  N  E  A  V  L  I  E  N  L  E  I  F  R  K  N  G  F  D
```

MATCH WITH FIG. 3K

MATCH WITH FIG. 3J

FIG. 3K



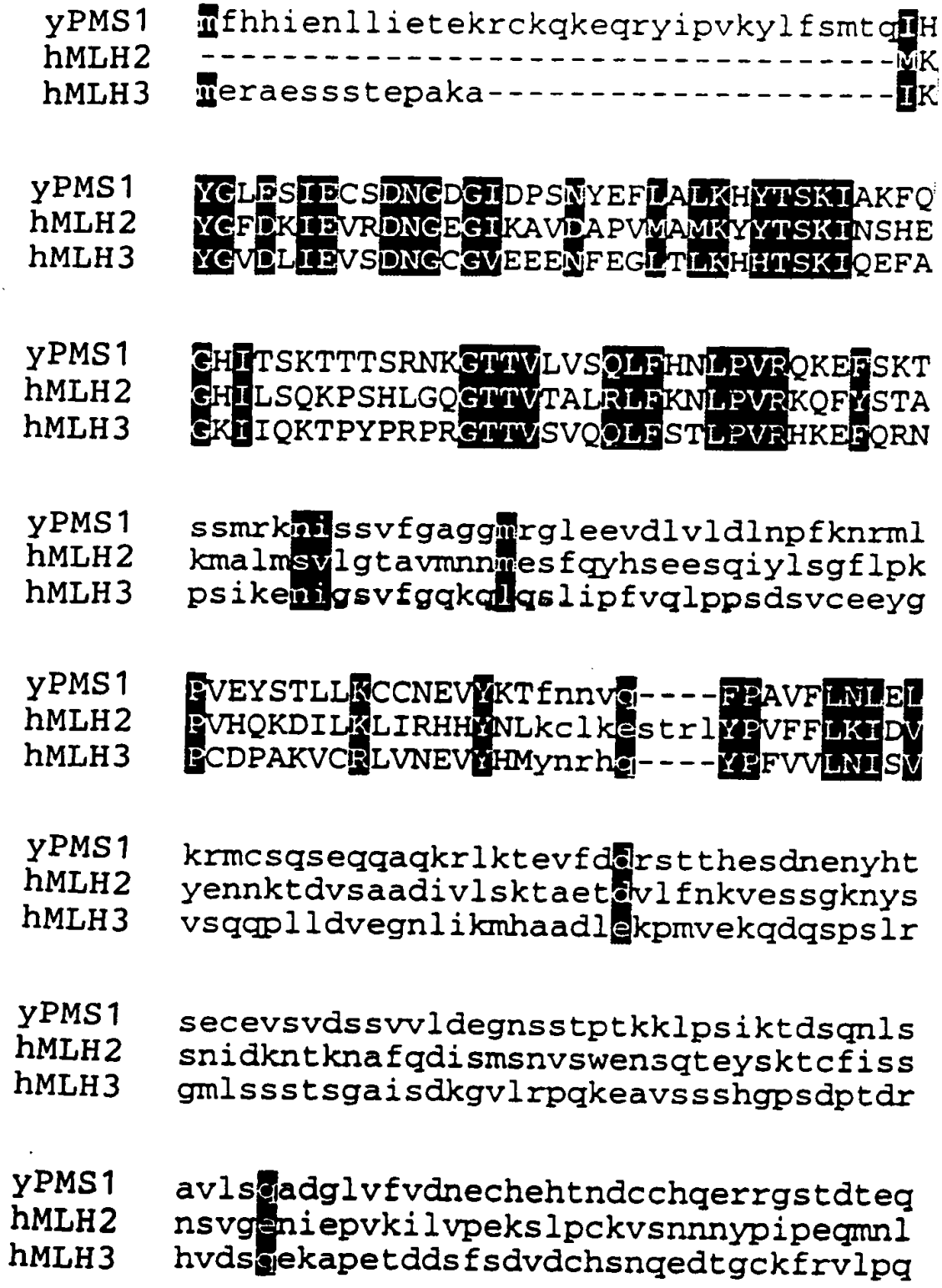
MATCH WITH FIG. 3L

MATCH WITH FIG. 3K

```
R  K  S  V  M  I  G  T  A  L  N  T  S  E  M  K  K  L  I  T
2500                2520                2540
CACATGGGGGAGATGGACCACCCCTGGAACTGTCCCCATGGAAGGCCAACCATGAGACAC
---+---------+---------+---------+---------+---------+------
GTGTACCCCCTCTACCTGGTGGGGACCTTGACAGGGGTACCTTCCGGTTGGTACTCTGTG
H  M  G  E  M  D  H  P  W  N  C  P  H  G  R  P  T  M  R  H
2560                2580                2600
ATCGCCAACCTGGGTGTCATTTCTCAGAACTGACCGTAGTCACTGTATGGAATAATTGGT
---+---------+---------+---------+---------+---------+------
TAGCGGTTGGACCCACAGTAAAGAGTCTTGACTGGCATCAGTGACATACCTTATTAACCA
I  A  N  L  G  V  I  S  Q  N  *
2620                2640                2660
TTTATCGCAGATTTTTATGTTTTGAAAGACAGAGTCTTCACTAACCTTTTTTGTTTTAAA
---+---------+---------+---------+---------+---------+------
AAATAGCGTCTAAAAATACAAAACTTTCTGTCTCAGAAGTGATTGGAAAAAACAAAATTT
2680                2700                2720
ATGAAACCTGCTACTTAAAAAAAATACACATCACACCCATTTAAAAGTGATCTTGAGAAC
---+---------+---------+---------+---------+---------+------
TACTTTGGACGATGAATTTTTTTTATGTGTAGTGTGGGTAAATTTTCACTAGAACTCTTG
2740
CTTTTCAAACC
---+-------
GAAAAGTTTGG
```

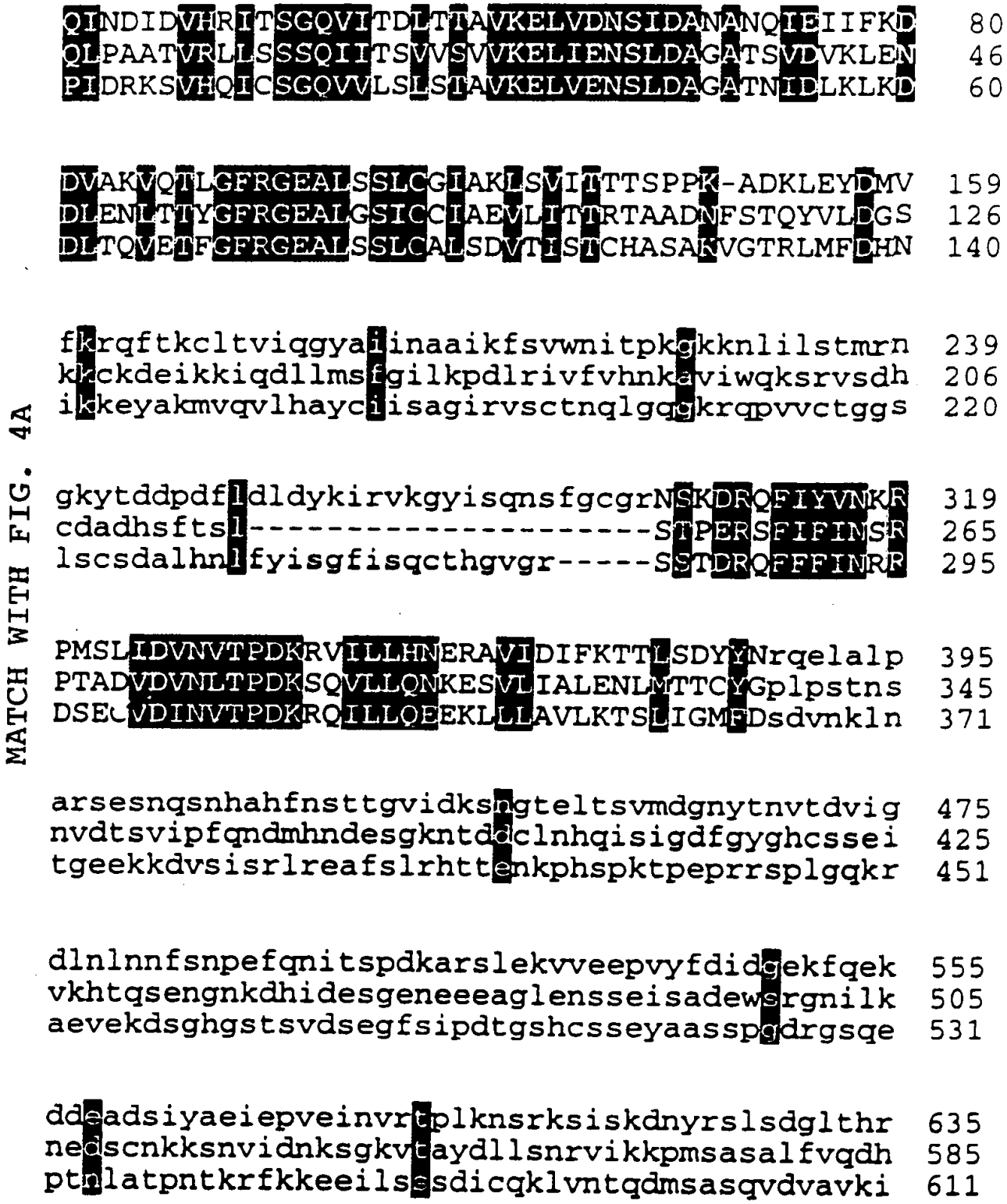
FIG. 3L

FIG. 4A



MATCH WITH FIG. 4C

FIG. 4B



MATCH WITH FIG. 4D

FIG. 4C

MATCH WITH FIG. 4A

```
yPMS1   kfedeileynlstknfkeiskngkqmssiiskrkse
hMLH2   rpqfliempktsledatlqieelwktlseeeklkye
hMLH3   nkkvvplsfsmsslakrikqlhheaqqsegeqnyrk

yPMS1   iivtrkvdnksdifivd   sdekynfetlqavtvf
hMLH2   hklktsisnqpkldellqsqiekrrsqnikmvqipf
hMLH3   nedifivdqhatdekynfemlqqhtvlqgqrliapq

yPMS1   srvkllslptskqtlfdlgdfnelihlikedgglrr
hMLH2   llnpyrveeallfkrllenhklpaeplekpimltes
hMLH3   tsknwtfgpqdvdelifmlsdspgvmc---------

yPMS1   ----------------------------------
hMLH2   vsitenyleiegmanclptygvadlkeilnailnrn
hMLH3   ----------------------------------

yPMS1   eldkpw--NCPHGRPTMRHLMEIrdwssfskdyei
hMLH2   hqfgneikECVHGRPFFHHLTYLpett--------
hMLH3   emdhpw--NCPHGRPTMRHIANLgvisqn------
```

MATCH WITH FIG. 4D

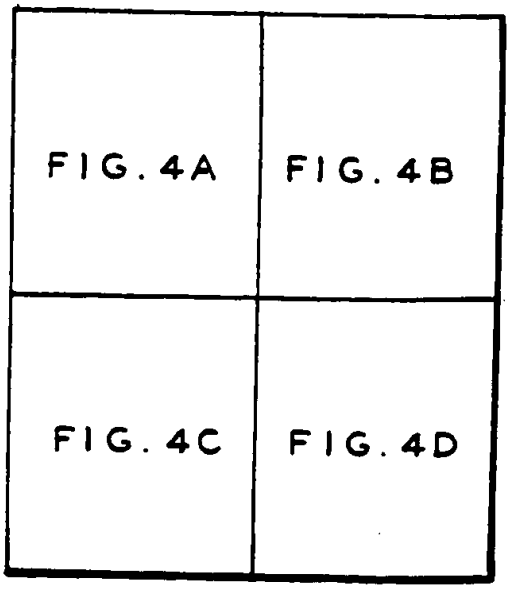


FIG. 4

FIG. 4D

MATCH WITH FIG. 4B

aqeniiknkdeledfeqgekyltltvskndfkkmevvgqfnlgf 715
ekatkdlerynsqmkraieqesqmslkdgrkkikptswnlaqk 665
frakicpgenqaaedelrkeisktmfaemeiigqfnlgfiitkl 691

MATCH WITH FIG. 4C

ksqkliipqqpvelsvidelvvldnlpvfekngfklkideeeefg 795
smknlkinfkkqnkvdleekdepclihnlrfpdawlmtsktevm 745
tlnltavneavlienleifrkngfdfvidenapvteraklislp 771

dni---------- 834
lfngshyldvlykmtaddqrysgstylsdprltangfkiklipg 825
---------- 798

-------RCSKIRSMFAMRACRSSIMIGKPLNKKTMTRVVHNLs 871
akevyecPPRKVISYLEGEAVRLSRQLPMYLSKEDIQDIIYRMk 905
-------PPSRVKQMFASRACRKSVMIGTALNTSEMKKLITHMg 835

904
932
862

FIG. 5A

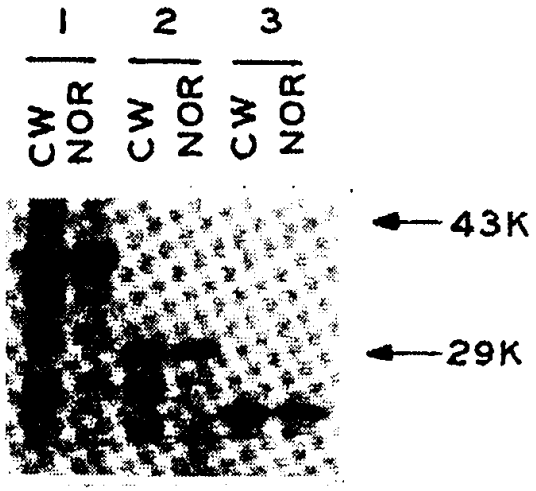


FIG. 5B

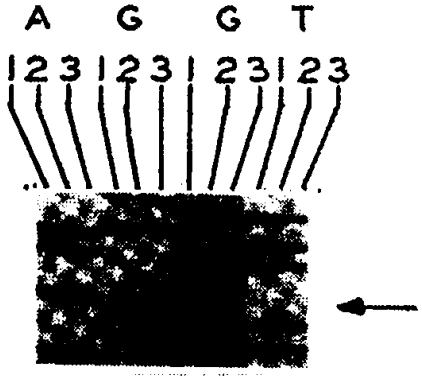


FIG. 6A

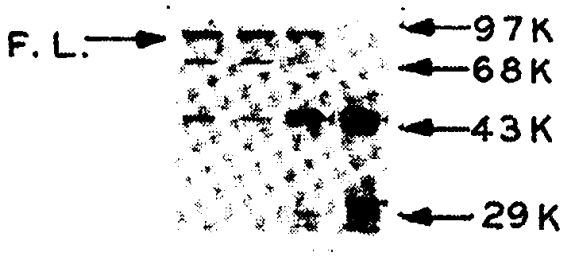
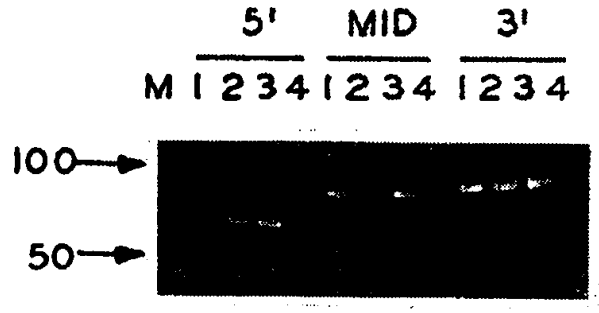


FIG. 6B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01035

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68; C12N 9/08; A61K 51/00; C07K 1/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Molecular and Cellular Biology, Volume 14, Number 1, issued January 1994, Prolla et al, "Dual requirement in Yeast DNA mismatch repair for MLH1 and PMS1, Two homologs of the Bacterial mutL gene," pages 407-415, especially page 407, column 2, line 3.	1-18, 21-23
P,Y	American Journal of Human Genetics, Volume 55, issued July 1994, Nystrom-Lahti et al, "Mismatch repair genes on Chromosome 2p and 3p account of a major share of Hereditary Nonpolyposis Colorectal Cancer families evaluable by linkage", pages 659-665, especially page 663, column 1, lines 9-13, and page 664, column 1, lines 19-30.	1-18, 21-23, 28-31

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 03 MAY 1995	Date of mailing of the international search report 22 MAY 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Dianne Rees, Ph.D. Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01035

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, Y	Science, Volume 265, issued August 1994, Prolla et al,"MLH1, PMS1 and MSH2 interactions during the initiation of DNA mismatch repair in yeast", pages 1091-1093, especially page 1091, column 1, and column 3, lines 1-5.	19, 20, 24-27
P, X ------ P, Y	Science, Volume 263, issued 18 March 1994, Papadopoulos et al, "Mutation of a *mutL* homolog in Hereditary Colon Cancer", pages 1625-1629, especially page 1626, column 1, paragraphs 1 and 2, figure 1, figure 3, and page 1627, column 3, paragraph 2, and p1628, notes: 13, 16, 17, 18, 20, 24, 25, 27.	5, 21-23, 29-31 ---------- 1-4, 6-20, 24-28
P, X ------ P, Y	Nature, Volume 368, issued 17 March 1994, Bronner et al, "Mutation in the DNA mismatch repair gene homologue *hMLH1* is associated with hereditary non-polyposis colon cancer, pages 258-261, especially page 259, figure 1, page 260, figure 2 and 3.	21, 31 ---------- 1-11, 14, 16-18, 25, 28-30
P, X ------ P, Y	Biochemical and Biophysical Research Communications, Volume 204, Number 3, issued 15 November 1994, Horii et al, "Cloning, Characterization and Chromosomal assignment of the human genes homologous to *PMS1*, a member of mismatch repair genes, pages 1257-1264, especially, page 1257, abstract, lines 10-14, page 1261, figure 2, and page 1262, figure 3.	7, 10, 13, 21, 31 ---------- 1-6, 8, 9, 11, 12-20, 22-30
Y	Cell, Volume 75, issued 16 December 1993, Leach et al, "Mutations of *mutS* homolog in Hereditary Nonpolyposis Colorectal Cancer", pages 1215-1225, especially page 1219, column 1, paragraph 3.	31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01035

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 192.1, 193.1; 530/300, 350, 358, 387.3, 388.21; 536/23.1, 23.4, 24.31

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

435/6, 192.1, 193.1; 530/300, 350, 358, 387.3, 388.21; 536/23.1, 23.4, 24.31

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

BIOSIS, MEDLINE, EMBASE, CAPLUS, HCA, USPATFULL, WPIDS, CANCERLIT, GENBANK, GENBANK, GENBANK-NEW, UEMBL (searched on seq IDs from related US case, US08187757, CRF disk was defective))
Search terms: human DNA repair (genes or proteins), mutator genes, mutL, hMLH1, hMLH2, hMLH3 , colon cancer, microsatellite instability, Haseltine, Prolla, Liskay

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-23, drawn to polynucleotides encoding polypeptides having the deduced amino acid sequences of hMLH-encoded proteins, their analogs or derivatives, vectors containing said polynucleotides, host cells genetically engineered with said vectors, process of growing said host cells.

II. Claims 24-27, drawn to polypeptides and methods of polypeptide production from host cells expressing hMLH genes.

III. Claims 28-31, drawn to a process for diagnosing cancer susceptibility comprising identifying mutations in hMLH1, hMLH2, hMLH3 and the human homolog of bacterial mutL.

An Election of Species for Groups I, II, and III is required wherein:
species A is drawn to hMLH1
species B is drawn to hMLH2
species C is drawn to hMLH3
and wherein Group III has an additional species:
species D, drawn to the human homolog of bacterial mutL.

These groups are separate and distinct from each other. Group I is drawn to products which are polynucleotides while Group II is drawn to products which are polypeptides and to a process of making said polypeptides. The products of Groups I and II have different structural and biochemical properties and may be used in distinctly different processes. Polynucleotides may be used as probes in linkage analyses, and DNA-based genetic therapy while polypeptides may be used in protein-based therapies. While the product Group I is linked to the process of Group II these do not share a common special technical feature according to PCT Rule 13.2 as "analogs, derivatives and variants" of group I are known in the art (Horii et al, Biochem. Biophys. Res. Commun., 28 November 1994). For the same reasons the product of Group I is also not technically linked to the process of Group III.

Species A-C (Groups I and II) and A-D (Group II) do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2 "the commonly shared structure" does not "constitute a structurally distinctive portion in view of the prior art", i.e. in view of Horii et al. 1994. Further the nonobvious differences in sequence structures between these genes render these genes structurally and functionally distinct. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.